DISSERTATION

on

EVALUATION OF HAEMATOLOGICAL SCORING SYSTEM IN EARLY DIAGNOSIS OF NEONATAL SEPSIS

submitted in partial fulfillment of the requirements for the degree of

Doctor of Medicine(BRANCH-III)

M.D. PATHOLOGY

THE TAMIL NADU DR. M.G.R. MEDICAL UNIVERSITY
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This is to certify that the dissertation titled **"EVALUATION OF HAEMATOLOGICAL SCORING SYSTEM IN EARLY DIAGNOSIS OF NEONATAL SEPSIS",** is a bonafide work done by **Dr.V.BRIJIN MARY**, Post Graduate Student, Department of Pathology, Tirunelveli Medical College, Tirunelveli – 627011, in partial fulfilment of the university rules and regulations for the award of MD DEGREE in PATHOLOGY BRANCH-III, under my guidance and supervision, during the academic period from 2016 to 2019.

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I hereby certify that this dissertation entitled "**EVALUATION OF HAEMATOLOGICAL SCORING SYSTEM IN EARLY DIAGNOSIS OF NEONATAL SEPSIS"** is a record of work done by **Dr.V.BRIJIN MARY**, in the Department of Pathology, Tirunelveli Medical College, Tirunelveli, during her postgraduate degree course period from 2016- 2019. This work has not formed the basis for previous award of any degree.

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THE FOLLOWING DOCUMENTS WERE REVIEWED AND APPROVED

1.	TIREC Application Form	☐	
2.	Study Protocol	☐	
3.	Department Research Committee Approval	☐	
4.	Patient Information Document and Consent Form in English and Vernacular Language	☐	
5.	Investigator's Brochure		☐
6.	Proposed Methods for Patient Accrual Proposed	☐	
7.	Curriculum Vitae of the Principal Investigator	☐	
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11.	DCGI/DGFT approval	☐	
12.	Clinical Trial Agreement (CTA)	☐	
13.	Memorandum of Understanding (MOU)/Material Transfer Agreement (MTA)	☐	
14.	Clinical Trials Registry-India (CTRI) Registration	☐	

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CERTIFICATE – II

This is certify that this dissertation work title **"EVALUATION OF HAEMATOLOGICAL SCORING SYSTEM IN EARLY DIAGNOSIS OF NEONATAL SEPSIS"** of the candidate **Dr.V.BRIJIN MARY** with registration Number **201613302** for the award of **M.D.** Degree in the branch of **PATHOLOGY (III).** I personally verified the urkund.com website for the purpose of plagiarism check. I found that the uploaded thesis file contains from introduction to conclusion page and result shows **17percentage** of plagiarism in the dissertation.

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LIST OF ABBREVIATIONS USED	
BPI	Bacteriocidal Perimeability Increasing protein
CAM	Cell Adhesion Molecule
CBC	Complete Blood Count
CD	Cluster Differentiation
CONS	Coagulase Negative Staphylococci
CRP	C-Reactive Protein
DAMP	Damage Associated Molecular Patterns
DIC	Disseminated Intravascular Coagulation
EDTA	Ethylene Diamine Tetra Acetic acid
EOS	Early Onset Sepsis
GBS	Group B-Streptococcus
G-CSF	Granulocyte Colony Stimulating Factor
GM-CSF	Granulocyte macrophage Colony Stimulating Factor
HMGB-1	High Mobility Group Box-1
HSPS	Heat shock Protein
HSS	Haemotological Scoring System
I:M	Immature to Mature Neutrophil
I:T	Immature to Total Neutrophil
IFN	Interferon
IL	Interleukin
LBW	Low Birth Weight
LOS	Late Onset Sepsis
LPS	Lipo Polysacchride
MODS	Multi Organ Dysfunction Syndrome
NICU	Neonatal Intensive Care Unit
NLR	Nod-Like Receptors
NPV	Negative Predictive Value

NRN	Neonatal Research Network
PAMP	Pathogen Associated Molecular Patterns
PCT	Procalcitonin
PMN	Polymorphonuclear Neutrophils
PPV	Positive Predictive Value
PRR	Pattern Recognition Receptors
P-value	Probabililty Value
RAGE	Receptor for Advanced Glycation End Products
RBC	Red Blood Cells
SIRS	Systemic Inflammatory Response Syndrome
TLC	Total Leucocyte Count
TLR	Toll-Like Receptors
TNF	Tumor Necrosis Factor
VLBW	Very Low Birth Rate
WBC	White Blood Cells

INTRODUCTION

Neonatal sepsis is defined as a disease with positive blood culture during the first month of life .It is more common in developing countries compared with developed countries.(1) Neonatal sepsis is associated with a mortality rate that ranges from 13 to 60% in spite of improved antibiotic therapy and care.(2)

Neonatal sepsis is a life-threatening condition, but treatable if diagnosed early. Unfortunately, the early warning signs and symptoms are often nonspecific and can easily be confused with those from non-infective causes. These nonspecific signs and symptoms make it difficult to establish an early clinical diagnosis. The antibiotic therapy is usually initiated based on the clinical suspicion which may result in overtreatment ultimately leading to emergence of multi drug resistant organisms. In addition, the high cost of antibiotics overburdens the already underprivileged parents. (3)

Blood culture is still considered to be the 'gold standard' for diagnosis of septicemia however, its accuracy has been questioned because of spurious positive results due to contamination and negative blood cultures in fatal generalized bacterial infections. The yield of a positive blood culture ranges from 8-73% as shown in various studies.(3) Moreover, the technique of blood culture is time consuming and demands

a well equipped laboratory which is not available in most of the community hospitals.

When blood and other sterile site cultures are negative, but the infant manifests signs consistent with infection they may be considered to have "clinical" sepsis. Importantly, a positive blood culture is not required to meet the consensus definition for sepsis in adults and children .(4) Therefore, there is a need for a test that is cheap ,easily performed with quick availability of reports. An ideal diagnostic test for neonatal sepsis should have maximum sensitivity and specificity. In recent years, various investigators have evaluated some highly sensitive and specific inflammatory markers to diagnose neonatal sepsis.(3) Although these markers are sensitive and specific, they are sophisticated and expensive and impractical for developing countries.(3)

Various cheap but reliable laboratory tests have been evaluated for the diagnosis of systemic infection in neonates **.** The complete blood count (CBC) with the various neutrophil parameters and C-reactive protein (CRP) are the most frequently used.(3) The present study is to evaluate the usefulness of the seven hematological parameters of Rodwell HSS , as an early indicator of neonatal septicemia as it is a simple bed-side test which can be done within a short time before putting the neonate on antibiotic therapy.

AIM & OBJECTIVES OF THE STUDY

1) To study the hematological parameters including the changes seen in the peripheral smears of neonates clinically suspicious of having sepsis.
2) To categorise the hematological findings according to Rodwell's Hematologic Scoring System.
3) To correlate these hematologic parameters with blood culture and C-reactive protein level in the serum.

REVIEW OF LITERATURE

HISTORICAL PERSPECTIVES

Sepsis historically has been a condition that is difficult to identify and diagnose. As far back as 100 BC, Marcus Terentius Varro, the ancient Roman scholar and writer (116 BC–27 BC), has quoted as noting that "small creatures, invisible to the eye, fill the atmosphere, and when breathed through the nose cause dangerous diseases." (5)

The description of sepsis was given by the historian, Niccolo Machiavelli (1469–1527), as reported in his treatise, "The Prince", in 1513. Early in the book, he stated that, "hectic fever, at its inception, is difficult to recognize but easy to treat; left unattended, it becomes easy to recognize and difficult to treat". Although hectic fever is not the name by which we know sepsis now, the description of a disease that is difficult to recognize in its early stages, and more difficult to treat in its later stages is a clear description of the more severe forms of sepsis.

In an attempt to better clinically understand sepsis, in the past century, a variety of definitions have been developed. Among the earliest concepts was to consider sepsis as a systemic host response to an infection.(5)

DEFINITION OF SEPSIS

National Neonatal Forum (NNF) of India has defined neonatal sepsis as follows:(6)

1.Probable (clinical) sepsis:

It is found in an infant having a clinical picture suggestive of septicemia if any one of the following criteria are present:

- ✓ Existence of predisposing factors:
  - ❖ Maternal fever
  - ❖ Foul smelling liquor
  - ❖ Prolonged rupture of membranes (>24 hrs).

The septic screen would be positive due to the presence of two of the four parameters namely,

- ❖ TLC (< 5000/mm)
- ❖ band to total polymorphs nuclear cells ratio of >0.2
- ❖ absolute neutrophil count < 1800/ml
- ❖ C-reactive protein (CRP) >1mg/dl
- ❖ micro ESR > 10 mm-first hour.

- ✓ Radiological evidence of pneumonia.

2. Culture positive sepsis

In an infant having a clinical picture suggestive of septicemia, pneumonia or meningitis, if any one of the following criteria are found:

- ✓ Isolation of pathogens from blood or CSF or urine or abscess
- ✓ Pathological evidence of sepsis in autopsy.(6)

EVOLUTION OF PATHOGEN IN SEPSIS

The causative organisms for sepsis have evolved over many years. Originally sepsis was described, and considered to be, a disease specifically related to Gram-negative bacteria. This is because sepsis was considered to be a response to endotoxin – a molecule that was thought to be relatively specific for Gram-negative bacteria. In fact, some of the original studies of sepsis bore out that Gram-negative bacteria were among the most common causes of sepsis(7).

This resulted in a number of trials that focused on Gram- negative therapies, and even highly specific therapies for endotoxin, which were felt to be potentially useful treatments for sepsis. We now recognize that sepsis may occur from any bacteria, as well as from fungal and viral organisms. More recent epidemiology studies reveal that Gram-positive bacteria have become the most common cause of sepsis in the past 25 years.(7)

TYPES OF SEPSIS

- **Early onset sepsis**
- **Late onset sepsis**

Based on the time of onset and mode of infection, sepsis is of following types: early onset sepsis (EOS), caused by maternal intrapartum

transmission of invasive organisms and diagnosed by means of positive blood cultures during the first 7 days of life or during the first 72 hours of life in the case of VLBW (very low birth weight) infants and late-onset sepsis (LOS) when infection is demonstrated in blood and cerebrospinal fluid cultures after 7 days from delivery, caused by postnatal nosocomial or community sources of the pathogen.(8)

Early onset sepsis (EOS)

EOS is due to infections occurring during the intrapartum period or just before delivery, due to "vertical transmission". The incidence is 1 to 2 per 1000 live newborns, reaching a mortality rate of 3% among term newborns, and 16% in very low birth weight infants.(8) Babies can become ill before or during labor due to an ascending infection caused by bacterial colonization of the maternal perineum or due to the direct contact between these microorganisms and the body of the newborn during delivery. Other causes being hematogenous transmission of infectious agents and chorioamnionitis are able to induce early onset sepsis. Aspiration and digestion of infected amniotic fluid in utero or infected secretion in the birth canal can effectively produce pneumonia or sepsis.(8)

Late Onset Sepsis (LOS):

Late onset sepsis is due to microorganisms acquired from the environment after delivery (nosocomial/ community acquired infections). The preterm infants, especially if very low birth weight are more prone for

late onset sepsis. There is significant increase in the survival rate due to recent advances in their management. Yet prolonged hospitalization, mechanical ventilation, use of invasive procedures and devices (i.e., intravascular catheters and endotracheal tubes), are all predisposing factors to LOS. Moreover, the immune system of newborns is immature which makes them more susceptible to sepsis. In the Neonatal Research Network (NRN) cohort, 70% of infections were associated with Gram-positive organisms; coagulase-negative staphylococci (CoNS) contributed 48%, Gram-negative 18% and fungal 12%.(9) In late preterm newborns (gestational age, 34-37 weeks) the incidence is about 6-10%. As the postnatal age increases mortality rate also increases, reaching 36% in newborns aged 8-14 days and 52% in those aged 15-28 days.(9)

RISK FACTORS FOR EARLY ONSET SEPSIS:

- Maternal risk factors
- Neonatal risk factors

Maternal risk factors:

- Premature birth (< 37 weeks),
- Premature rupture of membrane,
- Prolonged membranes rupture (> 18 hours),
- Maternal peripartum infection, and
- Low socioeconomic status.

Chan et al(10) further differentiated the predisposing factors into the following: maternal infection, maternal colonization, and risk factors for infection.

They defined maternal infection according to the following criteria:

- The presence of laboratory confirmed bacterial infection , bacteremia, amnionitis.
- Urinary tract infections
- Chorioamnionitis
- Documented by positive cultures of biologic fluids
- Positive polymerase chain reaction (PCR) at the level of the amniotic fluid
- Histopathologically confirmed chorioamnionitis
- Clinical signs of infection like [intrapartum maternal fever, uterine tenderness, maternal tachycardia, malodorous vaginal discharge, elevated white cell count, elevated C-reactive protein (CRP), physician diagnosis of clinical chorioamnionitis].

Maternal colonization was determined if positive reproductive tract/genital bacterial cultures with or without signs or symptoms of infection were identified.

The major maternal risk factors included

- Prelabor rupture of membranes (rupture of membranes before the onset of labour at <37 weeks of gestation),

- Preterm prelabor rupture of membranes (rupture of membranes prior to onset of labour at < 37 weeks of gestation) and
- Prolonged rupture of membranes (duration of rupture of membranes > 8to24 hours or undefined).(10)

Neonatal Risk Factors

Major neonatal factor which is able to promote early onset sepsis, is the alteration of the innate immune response. As the adaptive response requires 5-7 days from delivery to develop, during this period infants are largely dependent on innate immune system, the respiratory and intestinal barriers and the skin, local immune cells such as macrophages, endothelium, epithelium, polymorphonuclear cells (PMN), and dendritic cells, antigen presenting immune cells (monocytes, macrophages, and dendritic cells), host defense proteins and peptides (complements, cytokines, chemokines, and coagulation proteins), as well as passively acquired immunoglobulin from the mother. Prematurity especially with LBW are associated with an incomplete maturation and function of the innate immune system resulting in an increased likelihood of infections (11).

Birth weight also determines a major susceptibility to early onset sepsis; preterm neonates, especially very low birth weight, showed

incidence rates > 10 times higher than those born at term with a total mortality of about one-third.

Other neonatal risk factors include male sex, neonatal Apgar scoring at 1 minute and at 5 minutes, wet lung, fetal distress, anemia, intraventricular hemorrhage, hypothermia, and metabolic disorders.

Microorganisms associated with EOS:

Early onset sepsis can be due to bacteria, fungi, viruses, or protozoa; bacterial infections are the most frequent causes. Streptococcus agalactiae and Escherichia coli are the bacteria most commonly involved, followed by Listeria monocytogenes, Streptococcus pyogenes, streptococci Viridans, Streptococcus pneumoniae, Haemophilus influenza, Staphylococcus aureus, Enterococci, and Pseudomonas aeruginosa. Streptococcus agalactiae (Lancefield GBS) still represents the pathogen mainly responsible for neonatal sepsis (70% of GBS diseases) and meningitis despite the use of intrapartum antibiotic prophylaxis. (10)

Microorganisms associated with LOS:

According to Neonatal Research Network data, about 70% of the first episodes of late onset sepsis are caused by Gram-positive bacteria; CoNS were the most common pathogens (68% of Gram-positive infections and 48% of all infections), followed by S. aureus (8%), Enterococcus species (3%), and GBS (2%). Gram-negative organisms were responsible

for 18% of LOS. The remaining 12% were caused by fungal organism, of which Candida albicans was the most represented (6%). (9)

PATHOPHYSIOLOGY OF SEPSIS

In the pathophysiology of sepsis, so many molecular and cellular events takes place as follows (12)

- Molecular signaling
- Cytokines, chemokines and adhesion molecule in sepsis
- Anti-inflammatory response
- Role of complement in host defense and sepsis
- Role of dysregulated coagulation in severe sepsis
- Role of neutrophil in sepsis
- Role of neutrophil in sepsis shock
- Role of monocytes,macrophages and dendritic cells in sepsis

Molecular signaling:

Once local barrier function has been compromised, pathogen recognition by local immune cells is the first step towards the development of an immune response. Recognition is initiated by the activation of pattern recognition receptors (PRRs) .Toll-like receptors are the PRRs which are present on cell surface or within multiple cell types. These TLRs recognize

both extracellular and intracellular pathogens by their signature microbial products known as pathogen associated molecular patterns (PAMPs).

Lipopolysaccharide (LPS) which are the endotoxins on gram negative bacteria is the prototypic PAMP and a key mediator of systemic inflammation, septic shock, multi-organ failure and death(12). LPS signals primarily through TLR4. Lipoteichoic acid is the PAMP on the gram positive bacteria which signals primarily through TLR2.The viral PAMPS such as double-stranded RNA signal through TLR3. Microorganisms often stimulate more than one TLR simultaneously allowing for initiation of a pathogen-specific host response.

Ligand binding with the receptor results in downstream production of cytokines and chemokines, also favors activation of other antimicrobial effector mechanisms. In the leukocytes of neonates up regulation of TLR2 and TLR4 occurs during Gram-positive and Gram-negative infection, respectively. In experimental animal models, dysregulation or overexpression of TLR4 is involved in the development of necrotizing enterocolitis, demonstrating the importance of TLRs in the initial immune response to pathogens and their role in neonatal sepsis and septic shock. Mutations have been identified in NLRs that are involved in the pathogenesis of Neonatal-Onset Multisystem Inflammatory Disease (13).

TLRs can also be activated by DAMPs (damage or danger associated molecular patterns) such as intracellular proteins or mediators released by dying or damaged cells in addition being activated by PAMPs. An important DAMP is high mobility group box-1 (HMGB-1), which is found to be involved in the progression of sepsis to septic shock.

HMGB-1 is produced by macrophages or endothelial cells stimulated with LPS or TNF-α and signals through TLR2, TLR4, and receptor for advanced glycation end products (RAGE). Important functions of HMGB-1 include cytokine production, activation of coagulation, and neutrophil recruitment.

HMGB-1 induces reactive nitrogen intermediates which mediates disruption of epithelial junctions within the gut, leading to increased bacterial translocation. The role of HMGB-1 and RAGE signaling in septic shock in human neonates has not been well studied, but has been linked to the pathophysiology of necrotising entero colitis in a preclinical model (14).

Heat shock proteins (Hsps) and uric acid are other DAMPs which contribute to the pathophysiology of septic shock. Hsps activate proinflammatory signaling through TLRs, regulate neutrophil function, and are elevated in septic adults and children. Elevated Hsp60 and Hsp70 measured within first 24 hours of pediatric intensive care unit admission

was associated with pediatric septic shock and significant association with death.

Uric acid can increase cytokine production, PMN recruitment, and dendritic cell stimulation and also serve as an antioxidant. Uric acid is reduced in the serum of septic neonates as compared to control neonates. The importance of DAMPs in neonatal sepsis and shock has yet to be determined.(13)

Cytokines, Chemokines, and Adhesion molecule in Sepsis

PRR stimulation leads to increased production of cytokines and chemokines which results in amplification of the innate response directed against the invading organisms. Pro-inflammatory cytokines that are elevated during sepsis and septic shock include interleukin (IL)-1β, IL-6, IL-8, IL-12, IL-18, interferon gamma (IFN-γ), and tumor necrosis factor-alpha (TNF-α). (15)

Production of pro-inflammatory cytokines leads to activation of endothelial cells, expression of cell adhesion molecules (CAMs) that facilitate leukocyte recruitment and diapedesis. Upregulation of CAMs such as ICAM, VCAM, L-selectins, P-selectins, E-selectins, and CD11b/CD18 during sepsis facilitates rolling and extravascular migration of leukocytes.(16)

In addition to CAM interactions, Chemokine gradients produced by endothelial cells and local macrophages are necessary for effective and

specific leukocyte attraction and accumulation. Without adequate leukocyte recruitment, there is increased risk for propagation from a local infection to a systemic infection. Although poor cellular chemotaxis in the neonate has been observed, it is not likely a result of reduced serum concentrations of chemokines.(18)

Anti-inflammatory Response:

Inflammatory homeostasis is needed in order to prevent systemic inflammatory response syndrome (SIRS) associated with multi-organ failure and death. The balance between anti and pro-inflammatory stimuli serves to govern the immune response to allow local pathogen containment and prevent systemic activation leading to excessive inflammatory damage though SIRS(17). To end this, simultaneous increase in anti-inflammatory cytokine production occurs during infection, with TGF-β, IL-4, IL-10, IL-11, and IL-13 countering the actions of pro-nflammatory cytokines (18)

Role of complement in host defense and sepsis

Complement is an important component of early innate immunity that promotes killing of bacteria through opsonization and direct microbicidal activity. Complement components also have chemotactic or anaphylactic activity that increases leukocyte aggregation and local vascular permeability at the site of invasion. Complement components also activate a number of other important processes such as coagulation,

proinflammatory cytokine production, and leukocyte activation(19). Dysregulation of complement activation may results in the untoward effects seen in neonates with severe sepsis or septic shock. Very premature neonates, havedecreased basal levels of complement proteins for both the alternative and classic pathways[13]. Additionally, complement- mediated opsonization is poor in premature neonates and limited in term neonates(20).

In addition to the initial inflammatory response and complement activation following pathogen recognition, presence of microbes result in increases in other innate proteins that have valuable immune function(21).

The components which serve to reduce bacterial load include collectins (e.g. surfactant proteins A and D), bacteriocidal permeability increasing protein (BPI), and phospholipase A2, lactoferrin, cathelicidins(22).

Acute phase reactant proteins such as CRP (opsonin), haptoglobin and lactoferrin , serum amyloid A , procalcitonin , and others increase during sepsis and provide useful ancillary immune functions.(19)

Role of dysregulated coagulation in severe sepsis:

A procoagulant state is developed in the microvasculature surrounding a focal site of infection and this is a natural host defense mechanism, trapping the pathogens and preventing further dissemination. Similar to the inflammatory response, if the pro-coagulant response to

infection remains unchecked, it favours disseminated intravascular coagulation (DIC) resulting in severe tissue distruction and organ damage(23). Neonates with early elevated ratios of serum proinflammatory to anti-inflammatory cytokines during sepsis have an increased risk of developing DIC. This finding is consistent with the elevated serum levels of IL-6 and high frequency of DIC seen with disseminated herpes simplex viral infection (24).

Role of the neutrophils in sepsis

Neutrophils (Polymorphs)

Neutrophils originate from bone marrow stem cells and are released into circulation under the influence of granulocyte colony‑stimulating factor (G‑CSF) and granulocyte‑macrophage CSF (GM‑CSF) under baseline conditions.(25) The size is about 10-15 μm.

Neutrophils have dense nucleus with two to five lobes, and a pale cytoplasm with an irregular outline containing many fine pink azurophilic or grey blue granules . The lifespan of neutrophils in the blood is only 6 – 10 hours.

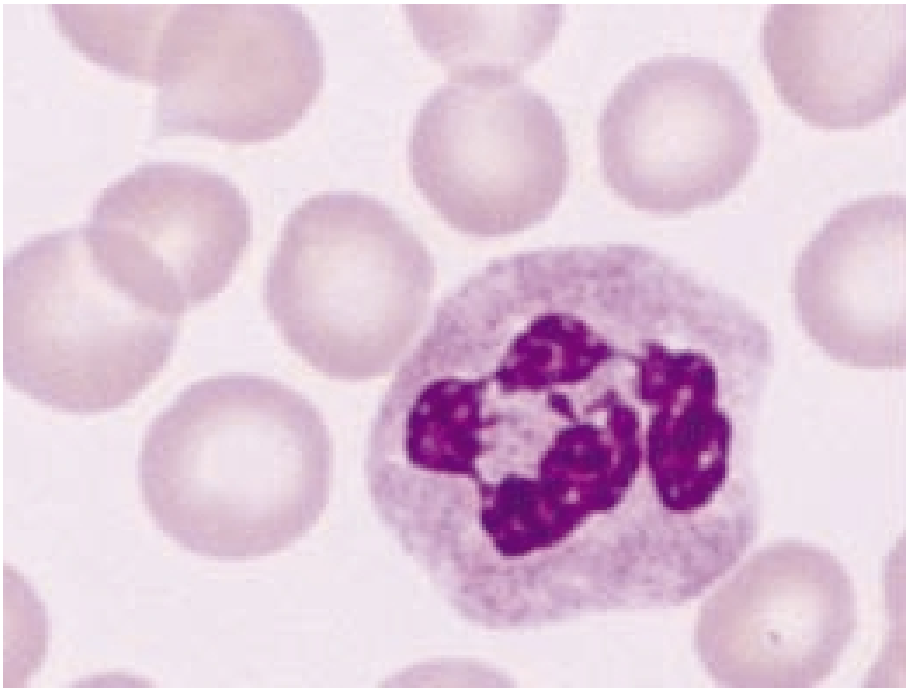

Fig i) Neutrophil showing dense nucleus with 4 lobes, and a pale cytoplasm with an irregular outline containing many azurophilic granules. (Adapted from Hoffbrand 6th edition, chapter-8)

Neutrophil precursors

Normally, neutrophil precursors do not appear in normal peripheral blood but are present in the marrow. The earliest precursor is the myeloblast, which is variable in size having large nucleus with fine chromatin and usually two to five nucleoli. The cytoplasm is basophilic and no granules are seen in the cytoplasm. The normal bone marrow contains up to 5% of myeloblasts. Myeloblasts divide and give rise to promyelocytes which are slightly larger than myeloblast and have developed primary granules in the cytoplasm. These cells then divide and differentiate to myelocytes which have secondary granules. The nuclear

chromatin is now more condensed and nucleoli are not visible. The myelocytes undergo cell division and differentiation in to metamyelocytes. Metamyelocytes are nondividing cells, which have an indented or horseshoe shaped nucleus and a cytoplasm filled with primary and secondary granules.

Neutrophil forms between the metamyelocyte and fully mature neutrophil are termed ‘band /stab/juvenile’ forms. These cells may occur in normal peripheral blood. They do not contain the clear, fine filamentous connections between nuclear lobes that is seen in mature neutrophils.(26)

Appearance of granules during neutrophil maturation

Promyelocyte and the myelocyte stages produces a distinct type of secretory granules: azurophils (dark granules) are produced only during the promyelocyte stage; specific granules (light granules) are produced during the myelocyte stage. The metamyelocyte and band forms are nonproliferating stages that develop into the mature polymorphonuclear neutrophil characterized by a multilobulated nucleus and cytoplasm containing primarily glycogen and granules. Both nonspecific azurophilic granules and specific granules persist throughout these later stages. (27)

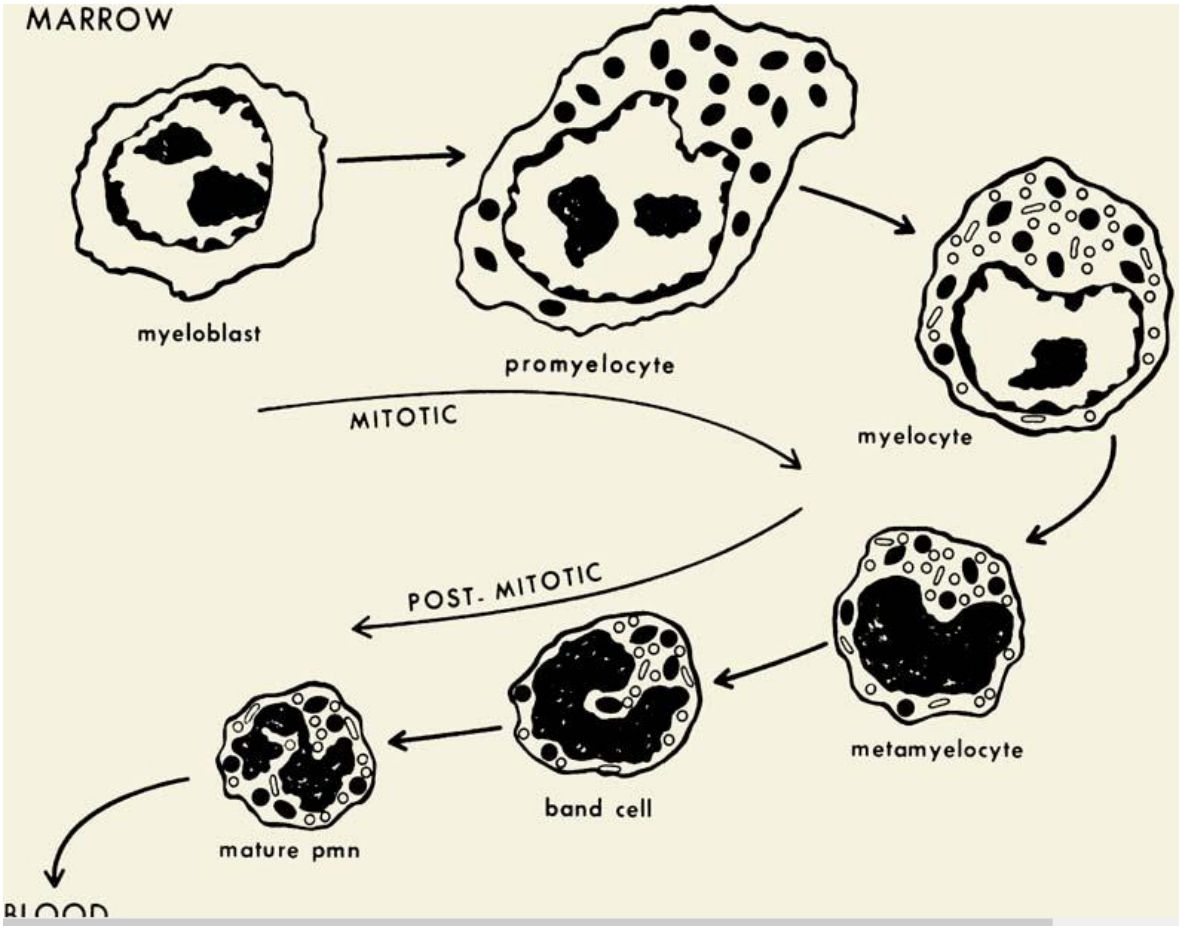

Fig ii) Appearance of granules during neutrophil maturation

(Modified from Bainton DF, et al. The development of neutrophilic PMN leukocytes in human bone marrow: origin and content of azurophil and specific granules. J Exp Med 1971;134:907. (Adapted from wintrobe clinical hematology 12th edition)

Neutrophils release the proteins in three types of granules by a process called degranulation. The contents of these granules have antimicrobial properties, and help combat infection.(28)

Granule type	Protein
Azurophilic granules (or "primary granules")	Myeloperoxidase, bactericidal/permeability-increasing protein (BPI), defensins, and the serine proteases ,neutrophil elastase and cathepsin G
Specific granules (or "secondary granules")	Alkaline phosphatase, lysozyme, NADPH oxidase, collagenase, lactoferrin, histaminase, and cathelicidin
Tertiary granules	Cathepsin, gelatinase and collagenase

Role of the neutrophil in septic shock

Neutrophil or polymorphonuclear leukocyte (PMN) are the most important means of early innate cellular defense against bacterial invasion in neonates .When compared to adult neutrophils, neonatal PMNs exhibit deficits both quantitatively and qualitatively(29). Three aspects of PMN functions which results in severe neonatal sepsis and septic shock are neutropenia, decreased deformability, and delayed apoptosis.

Under normal conditions, large numbers of the peripheral blood neutrophils enter sites of bacterial infection by first adhering to activated endothelial cells and then migrating along a gradient of chemotactic factors. These chemotactic factors are produced at the local site of

infection. Neutrophils use Toll-like receptors (TLR-2 or TLR-4) to interact with pathogen-associated molecular patterns on bacteria to phagocytize and eliminate the pathogens. In contrast, neutrophils from septic patients have increased expression of surface integrins, which promote firm adhesion to endothelial cells. As a consequence, the neutrophils remain bound more tightly to the endothelial cells and fail to migrate appropriately into the site of the bacterial infection.(14)

Rapid depletion of neonatal marrow PMN reserves during infection can lead to neutropenia with consequent impaired antimicrobial defenses and significantly increased risk for death(30).Neutropenia is commonly associated with Gram-negative sepsis in neonates(31).

Prematurely released immature neutrophil forms (bands) have a greater dysfunction when compared to mature neonatal neutrophils and these immature neutrophils predispose to further adverse outcomes(32). During sepsis ,PMN respiratory burst activity is also suppressed which contributes to poor microbicidal activity(33). PMNs of neonates have reduced deformability compared to adult PMNs. In addition to this, low blood pressure/flow state associated with septic shock, increases the risk of microvascular occlusion .(17) In the vascular spaces there will be irreversible aggregation of newborn PMNs, which leads to decreased diapedesis, rapid depletion of bone marrow reserves, vascular crowding, and compromised tissue perfusion all leading to organ dysfunction(34).

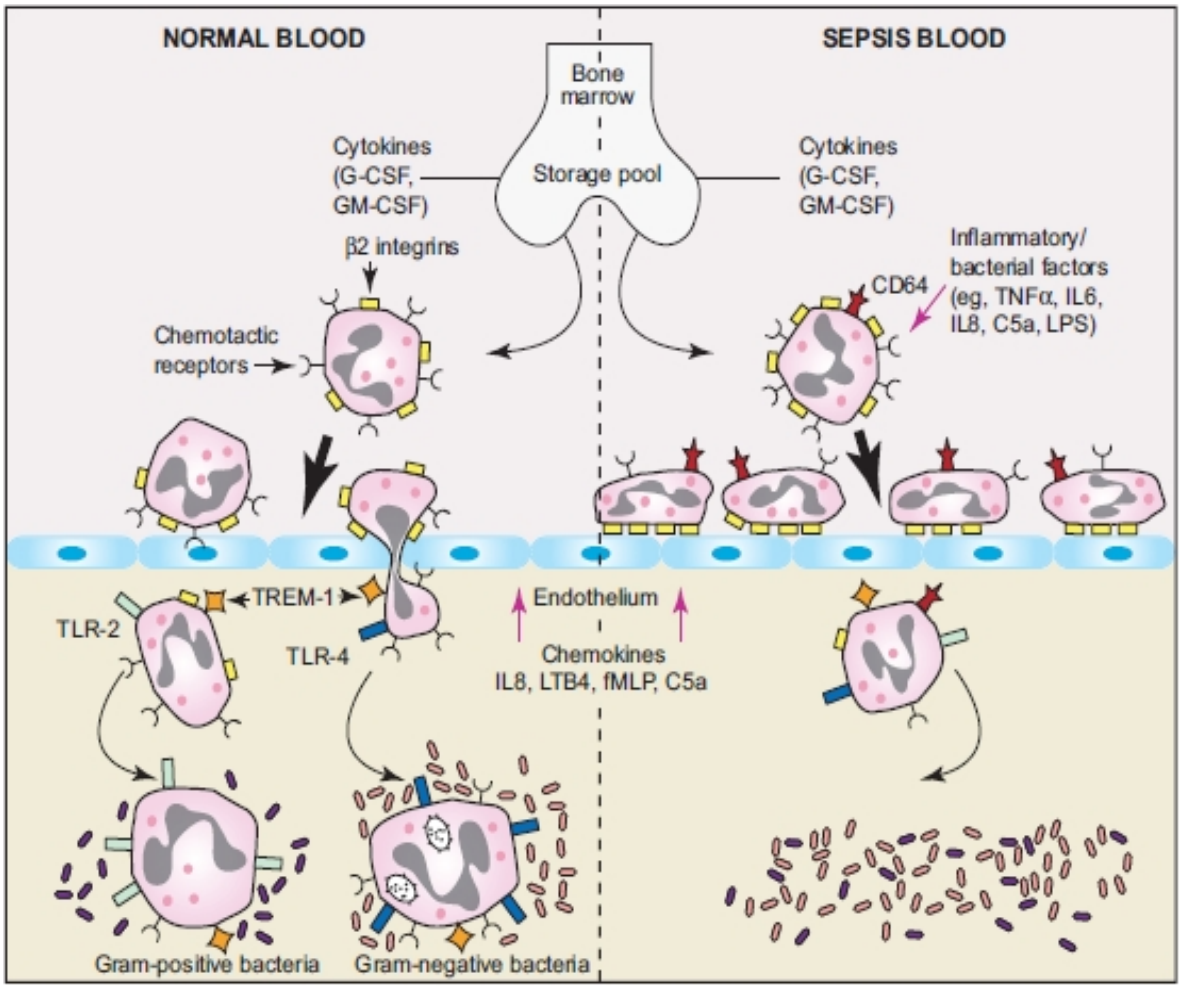

Fig iii) **Dysregulation of neutrophil recruitment to bacterial infection under normal conditions (left) and in sepsis (right).** {Redrawn from The Lancet, 368, Brown KA, Brain SD, Pearson JD, Edgeworth JD, Lewis SM, Treacher DF, Neutrophils in development of multiple organ failure in sepsis, 157–169, Copyright (2006),69 with permission from Elsevier. (Adapted from, "Pathophysiology of Sepsis", The American Journal of Pathology)}

Neutrophils are essential for defending against the pathogens, but they can also cause significant tissue damage and thus play a role in progression from sepsis to multi-organ system dysfunction. PMNs produces reactive oxygen species, nitrogen intermediates and proteolytic

enzymes which are released extracellularly, via activation of membrane associated- NADPH oxidase. Extracellular release of these reactive intermediates and enzymes favours destruction of non-phagocytized bacteria as well as local tissue destruction.(12) Increased levels of neutrophil elastase , neutrophil activators like urokinase plasminogen activator, and urokinase plasminogen activator receptor have been described in infected neonates.(16) When comparing adult PMNs with neonatal PMNs, neonatal PMNs exhibit delayed apoptosis as well as sustained capacity for activation and cytotoxic function that contributes to tissue damage (35), this is due to prolonged activation of nuclear factor k ß and reduced caspase 3 levels .Neutrophil mediated damage leads to endothelial and lung injury (including surfactant inactivation) in addition to other organ dysfunction.(36)

Role of monocytes, macrophages, and dendritic cells in sepsis:

Monocytes, macrophages, and dendritic cells enhance the cellular recruitment through production of inflammatory mediators, phagocytosis and killing of pathogens, and antigen presentation to cells of the adaptive immune system. Important substances produced by stimulated monocytes that may contribute to septic shock include complement components, cytokines (both pro and anti-inflammatory), coagulation factors, and extracellular matrix proteins(37). The role of NK cells in neonatal bacterial sepsis is incompletely defined.

Activated or damaged endothelium favours a prothrombotic environment that can result in local microvascular occlusion or progress to DIC (38). Endothelial cell apoptosis, detachment from the basal lamina, and alterations in vascular tone combine to promote capillary leak of proteins and fluid leading to hypovolemia and shock(39)

Multi-organ dysfunction syndrome(MODS)

Septic shock that leads to multi-organ failure or MODS carries a worse prognosis. Poor cardiac output and microcirculatory failure, combined with formation of microthrombi and DIC, favours compromised perfusion to the kidney, liver, gut, and CNS(40). Recent studies suggest that the mechanism of organ failure in sepsis may relate to decreased oxygen utilization associated with mitochondrial dysfunction in addition to poor oxygen delivery to tissues(41). Many other organ systems can be compromised in the setting of septic shock. Pulmonary complications include acute respiratory distress syndrome, secondary surfactant deficiency(42), pulmonary edema, pneumonia. Endocrine abnormalities may include adrenal insufficiency associated with refractory hypotension and altered thyroid function(43). Lymphocyte loss secondary to thymic involution and splenocyte apoptosis may also be present and may lead to a state of immune compromise following the acute phase of sepsis(44) (45)

Finally, sepsis can lead to nutritional and metabolic consequences. There is increased energy expenditure and oxygen consumption (46)with

decreased mitochondrial oxidative function precipitated by hypoxia and the presence of damaging free radicals ultimately may lead to impaired growth and energy failure(47)

CLINICAL MANIFESTATIONS

Clinical manifestations of neonatal sepsis is highly variable depending on the virulence of pathogens and on the mechanisms of host defense. Term neonates may have elevated, normal, or depressed body temperature; preterm newborns often present with low temperatures and irregular fluctuations. Diagnosis is particularly difficult in preterm and LBW infants due to the immaturity of the immune system that makes signs and symptoms misleading.(48) Motor functions are characteristically reduced; delayed weight gain, pale skin, and reduction of activity mainly movements, eating, crying, are often observed. Cyanosis, apnea, tachycardia, bradycardia, and hypotension represent warning signs for severe and rapidly evolving forms as they can be considered precursors of shock (cold extremities, decreased femoral pulses, congestive heart failure, and even disseminated intravascular coagulation). Jaundice may sometimes be the only manifestation, preceding encephalopathy in severe cases.

All organs and systems may be affected; the central nervous system involvement can induce drowsiness, irritability, lethargy, convulsions, and

increased tension at the fontanelle level. Anorexia, regurgitation, abdominal distension, vomiting, diarrhea, and necrotizing enterocolitis are common symptoms of gastrointestinal lesions. Skin lesions are frequent; these include cutaneous and mucosal petechiae, impetigo, cellulitis and abscesses. Involvement of cardiovascular system in the form of myocarditis, pericarditis, endocarditis, heart failure, septic shock with thrombotic-hemorrhagic manifestations, urinary tract infections, osteomyelitis, and deep infections are also possible. System wise specific features as per NNF(National Neonatal Forum)are as follows.(49)

Respiratory System:

- Apnea
- Gasping
- Respiratory distress
- Tachypnea
- Chest indrawing

Central nervous system:

- Bulging anterior fontanelle
- Blank look
- High-pitched cry
- Excessive irritability
- Coma

- Seizures
- Neck retraction.
- Presence of these signs should raise clinical suspicion of meningitis

Cardiovascular System:

- Hypotension.
- Poor perfusion.

A recent study emphasized the value of early diagnosis of sepsis using analysis of heart rate characteristics on ECG monitoring. Griffin et al found that abnormal heart rate characteristics such as reduced variability and transient decelerations occurred 24 hours prior to onset of symptoms in sepsis and sepsis like illness.(50) Another group found that sample asymmetry of RR interval increased in the 3-4 days preceding sepsis with the steepest increase in the last 24 hours. These tests may prove helpful in starting the therapy long before the baby shows signs of deterioration.(51)

Gastrointestinal System:

- Feed intolerance
- Vomiting
- Diarrhea
- Abdominal Distension
- Paralytic ileus, Necrotising enterocolitis.

Hepatic System:

- Hepatomegaly
- Direct hyperbilirubinemia

(Infants with the onset of jaundice after 8 days of age or with direct hyperbilirubinemia were more likely to have urinary tract infection). (9)

Renal System:

- Sings of acute renal failure

Hematological Sings:

- Bleeding
- Petechiae and purpura

Skin:

- Multiple pustules
- Sclerema
- Mottling
- umbilical redness and discharge

DIAGNOSIS OF SEPSIS

Serum inflammatory biomarkers (acute-phase reactants, inflammatory cytokines) may be helpful, no single laboratory test is sufficient for the absolute diagnosis. For this reason, delays may occur in the early identification of neonatal sepsis. This delay in identifying affected neonates may lead to prolonged and unnecessary therapy, with the

emergence of resistant microorganisms, increased health care spending, and especially a higher risk of complications such as cerebral palsy or intraventricular hemorrhage. In order to make a diagnosis, several clinical and hematological parameters are generally considered together, although the correct combination is not well-established.

Hematologic scoring system:

Rodwell et al formulated a hematologic scoring system (HSS), which was easy to perform and cost-effective, based on the following seven criteria:

- Total leukocyte count
- Total PMN count
- Elevated immature PMN count
- Elevated immature-to-total PMNs ratio
- Immature-to-mature PMNs ratio ≥ 0.3
- Platelet count $\leq 150{,}000/\ mm3$
- Pronounced degenerative changes in PMNs

A score > 2 means likelihood of sepsis, whereas ≤ 2 is related to 99% likelihood of its absence.(52)

Serum markers:

New leukocyte parameters like neutrophil and monocyte volume, conductivity, scattering, and volume distribution width may be useful in the differential diagnosis of newborn sepsis.(53) Macrophage, cytokines, which are produced in response to microorganism antigens ,which in turn stimulate the release of acute-phase reactants and the host inflammatory immune reactions. These are usually used in clinical practice as indicators of both early onset sepsis and late onset sepsis (54)

Serum markers are found to increase earlier than the changes seen in hematological parameters, hence serum markers play a pivotal role in the diagnostic process, allowing detection of sepsis and its severity. Also differentiation of bacterial from fungal and viral agents, and monitoring of response to therapy can be done using serum markers (55) .

TNF-α:

The TNF-α test showed moderate accuracy of the diagnosis of neonatal sepsis both in early-onset neonatal sepsis and in late-onset neonatal sepsis (56).

IL-6 and IL-8

IL-6 and IL-8 plasma concentrations are considered to be sensitive and specific for the prediction of neonatal sepsis. These indicators can be detected in blood early but their short half-life, of about 12 to 24 hours, limits their use in clinical practice.(57)

C reactive protein [CRP]

C reactive protein [CRP], a peptide synthesized by the liver in response to infection or inflammatory processes, was one of the best diagnostic marker of neonatal sepsis, with higher sensitivity and specificity than total PMN count and immature-to-total-PMN ratio.(58) However, it presents a low sensitivity during the early phases of infection because the time needed for release of CRP is about 6 hours. So serial determinations improve the diagnostic accuracy and are useful for evaluating the response to treatment.(59)

Granulocyte colony-stimulating factor

The granulocyte colony-stimulating factor was shown to have sensitivity of 95% and negative predicting value (NPV) of 99% in detecting infection in neonates of all gestational ages when a cut-off level of 200 pg/mL was used.(60)

Presepsin

Presepsin, a truncated form of soluble CD14, can be used as a reliable biomarker for late onset sepsis and treatment response in preterm infants.(62)

Procalcitonin (PCT)

Procalcitonin , a peptide produced by monocytes and hepatocytes in response to systemic inflammation, is more specific than CRP in bacterial infections(61) In neonatal sepsis, its concentration increases after 4 hours

from pro inflammatory action of bacterial endotoxins, reaching the peak after 6 to 8 hours, so a rise of PCT value is more precocious compared to CRP. In normal-birth-weight neonates, a PCT cut-off limit > 0.5 ng/mL indicates a two-fold probability of nosocomial sepsis, while a value > 2.4 ng/mL in infected very low birth weight infants suggests the need for an empirical antibiotic therapy.

Leucocyte Differentiation Antigen Markers

Leukocyte differentiation antigens, CD33, CD66b, and CD19, induced by inflammation secondary to bacterial infections, increase in preterm newborns with sepsis. In addition, an increased expression of PMN Fc-gamma receptor I (CD64) has been demonstrated in newborns during the early phase of an acute bacterial infection.

Weirich et al (62)proposed neutrophil CD11b as a precocious marker of neonatal infection.

Proteomics

Recent proteomics-based technologies provided novel biomarkers for identifying pregnancies at risk for intrauterine infection and prenatal fetal damage.(63)

Molecular genetics

Molecular genetic techniques can further help physicians in the diagnosis of neonatal sepsis by identifying specific fungal, bacterial and

viral genes in neonatal blood through amplification of target DNA/RNA fragments.(64)

Blood culture

Definitive diagnosis is still by microbiological culture examination of biological samples mainly on blood, urine, cerebrospinal fluid are considered the gold standard for the detection of bacteremia or fungemia, despite their limitations of low sensitivity (sepsis due to bacterial endotoxins induce negative cultures) and the time required for results (48 to 72 h), which can retard the beginning of antibiotic therapy and compromise the life of newborns.(65) The yield of a positive blood culture ranges from 8-73% as shown in various studies(66) Therefore, the need is for a test that is cheap , easily performed with quick availability of reports.

An ideal diagnostic test for neonatal sepsis should have maximum sensitivity and specificity. Although, inflammatory markers are sensitive and specific, they are sophisticated and very expensive and impractical for developing countries. Various cheap but reliable laboratory tests have been evaluated for the diagnosis of systemic infection in neonates**.** The complete blood count (CBC) with the various neutrophil parameters and C-reactive protein (CRP) are the most frequently used(67)

HEMATOLOGICAL PROFILE:

There are a variety of tests, as mentioned above which are helpful for screening of neonates with sepsis. The most widely used is the white blood cell count and differential count.

An absolute neutrophil count of < 1800 /cumm is an indicator of infection. Neutropenia is more predictive of neonatal sepsis than neutrophilia. Immature neutrophils (band forms, myelocytes, metamyelocytes) to total neutrophil ratio (I:T) > 0.20 means that immature neutrophils are over 20 percent of the total neutrophils because bone marrow pushes even the premature cells into circulation, to fight infection. Platelet count of less than 100000 per cu.mm, and toxic granules on peripheral smear are also useful evidences of infection. The micro-ESR may be elevated with sepsis and fall of > 15 mm during first hour indicates infection.

METHODOLOGY

The present study was conducted in the Department of Pathology, Tirunelveli medical college, Tirunelveli.

MATERIALS AND METHODS

Study Design

The study design was a cross-sectional study.

Study Area

Department of Pathology, Tirunelveli Medical College Hospital.

Source of Data

Analysis of the hematological parameters of 100 neonates with suspicion of sepsis and history of maternal infection admitted to the neonatal intensive care unit at Tirunelveli Medical College Hospital, Tirunelveli.

Duration of study

March 2017 to April 2018

Sample size

Studied 100 cases

Inclusion criteria

1. Neonates with features suggestive of sepsis:

   Fever

   Lethargy

   Poor feeding

   Need for supplemental oxygen

   Low APGAR score

2. Neonates with history of maternal infection:

   Maternal intrapartum fever >38ºC

   Premature rupture of membrane <37 weeks

   Prolonged rupture of membrane > 12 hrs

   Maternal UTI

All suspected cases of neonatal sepsis were included in the study.

Exclusion criteria

Neonates with

1. Major congenital anomaly
2. Inborn errors of metabolism
3. Administration of antibiotics prior to admission
4. Babies with respiratory distress syndrome

Neonates of mothers with pregnancy induced hypertension and asphyxia was excluded after detailed perinatal history and clinical examination.

Ethical committee approval

Study was conducted only after getting approval of the institutional ethical committee. A copy of approval is enclosed

Consent form

Neonates fulfilling the selection criteria were selected and their parents or legal guardians were briefed about the nature of the study and a written informed consent in regional language was obtained.

Data collection

Parents were interviewed regarding birth information, presenting complaints. A thorough clinical examination was conducted. These findings were recorded on a predesigned and pretested proforma. Blood samples of neonates suspicious of sepsis were collected to study the haematological parameters. Clinical history, physical findings and probable diagnosis were noted. Complete blood count was done using SYSMEX, a three part analyser which gives nineteen parameters per sample including total red cell count, total leucocyte count, differential count, haemoglobin concentration, packed cell volume, mean corpuscular haemoglobin, platelet count, mean platelet volume, platelet distribution

width. Standardisation, calibration of the instrument and processing of samples were done as per manufacturer instruction Leishman stained peripheral smears were examined by counting 200 WBCs for,

- Nucleated red blood cells,
- Differential counts,
- Absolute neutrophil count,
- Immature neutrophils,
- Toxic granulations in neutrophils and degenerative neutrophils.

CRP and blood culture was also done. The blood samples collected aseptically in EDTA vacutainers by peripheral venipuncture, heel prick peripheral smear received from NICU, Tirunelveli Medical College and Hospital, Tirunelveli were used in the study.

Procedure

Blood sample was obtained by peripheral venipuncture (3ml) and the sepsis workup including blood culture and routine blood count done. 2 ml of the blood collected was anticoagulated in EDTA for routine hematological investigations, other 1 ml blood was transferred to the conventional blood culture tube for culture and sensitivity study. After sterile precautions, peripheral smear was made by heel prick method and hematological scoring was done simultaneously.

The differential counts were performed on Leishman stained blood smears by counting 200 cells. A pathologist blinded to the infection status of the neonate reviewed the peripheral smears. Degenerative morphological changes in the neutrophils such as Dohle bodies, vacuolation and toxic granules were noted. the hematological findings were analysed according to the HSS of Rodwell et al.(68)

Parameters for assessment:

1) Total leucocyte count
2) Total neutrophil count
3) Immature neutrophil count
4) Immature to total neutrophil ratio
5) Immature to mature neutrophil ratio
6) Toxic granulations and degenerative neutrophils in peripheral smear.
7) Platelet count

The HSS assigns a score of one for each of the seven parameter found to be significantly associated with sepsis, with one expection. An abnormal total PMN count is assigned a score of two rather than one if no mature PMNs are seen on the blood smear.

Clinical details as well as CRP and culture results were compared with the haematological score .

Hematological scoring system

	CRITERIA	ABNORMALITY	SCORE
1	Total WBC count	≤5,000/µl	1
		≥25,000 at birth	
		≥30,000—12–24 h	
		≥21,000—Day 2 onwards	
2	Total PMN count	No mature PMN seen	2
		Increased/decreased	1
3	Immature PMN count	Increased	1
4	I:T PMN ratio	Increased	1
5	I:M PMN ratio	≥0.3	1
6	Degenerative changes in PMN	Toxicgranules / cytoplasmic vacuoles	1
7	Platelet count	≤150,000/µl	1

The normal values are

Total PMN count—1800–5400

Immature PMN count—600

Immature: Total PMN ratio—0.120

Immature: Mature PMN ratio—≥0.3

Interpretation of hematological scoring system(69)

SCORE	**INTERPRETATION**
≤2	Sepsis is unlikely
3 or 4	Sepsis is possible
≥5	Sepsis or infection is very likely

The absolute neutrophil count varies considerably in the immediate neonatal period and the normal ranges are available from Manroe's chart.(70) For very low birth weight infants ,the reference ranges are available from Mouzinho charts .(71)

C-Reactive protein test (Latex slide test method**)**

Place one drop of test serum within the circled area on the special slide to that add one drop of latex CRP reagent and mix with a disposable applicator stick and spread out to the edge of the test area. Rock the slide gently to and fro for two minutes and look for macroscopic agglutination under direct light source. Coarse agglutination indicates strong positivity and fine agglutination indicates weak positivity for C-reactive protein.

Blood culture methodology

One ml of blood was inoculated in conventional blood culture bottle containing 10 ml of glucose broth. Within six hours ,0.1 ml of this broth was aspirated with a sterile syringe and needle and is used to make subcultures on Nutrient agar, Blood agar and MacConkey's agar plates. The plates were then incubated at 37°C for 48 to72 hours. If growth does not occur, this process was repeated every 24 or 48 hours for isolation of bacteria or till two weeks. If the growth occurs, the colonies were identified macroscopically and microscopically. Finally, antibiotic sensitivity was tested for the cultured organism.

Statistical Analysis

Data were entered in MS Excel and analysis was done using SPSS 16.0. version. Descriptive statistics like Mean ± Standard deviation was

done for quantitative variables and proportion or percentage for categorical variables. Chi-square test was used to find out the association between categorical variables and student t test was done for quantitative variables. ROC analysis was done to find out the cut off values. Sensitivity, specificity, positive predictive value and negative predictive value were calculated to assess the validity of tests. A probability value (p value) of less than 0.05 was considered to be statistically significant.

OBSERVATION AND RESULTS

This cross-sectional study was conducted in the Department of Pathology, Tirunelveli Medical college, Tirunelveli. Neonates suspected to have sepsis admitted in the Sick Neonatal Intensive Care Unit, Tirunelveli Medical College Hospital, were studied. During the study period ,100 neonates were eligible based on the selection criteria, hence all eligible neonates were included in the study.

The data obtained was tabulated and analysis was done. The final observation and results were tabulated as below.

TABLE 1. GENDER DISTRIBUTION

GENDER	DISTRIBUTION (n=100)	
	NUMBER	PERCENTAGE
MALE	59	59.00
FEMALE	41	41.00
TOTAL	100	100.00

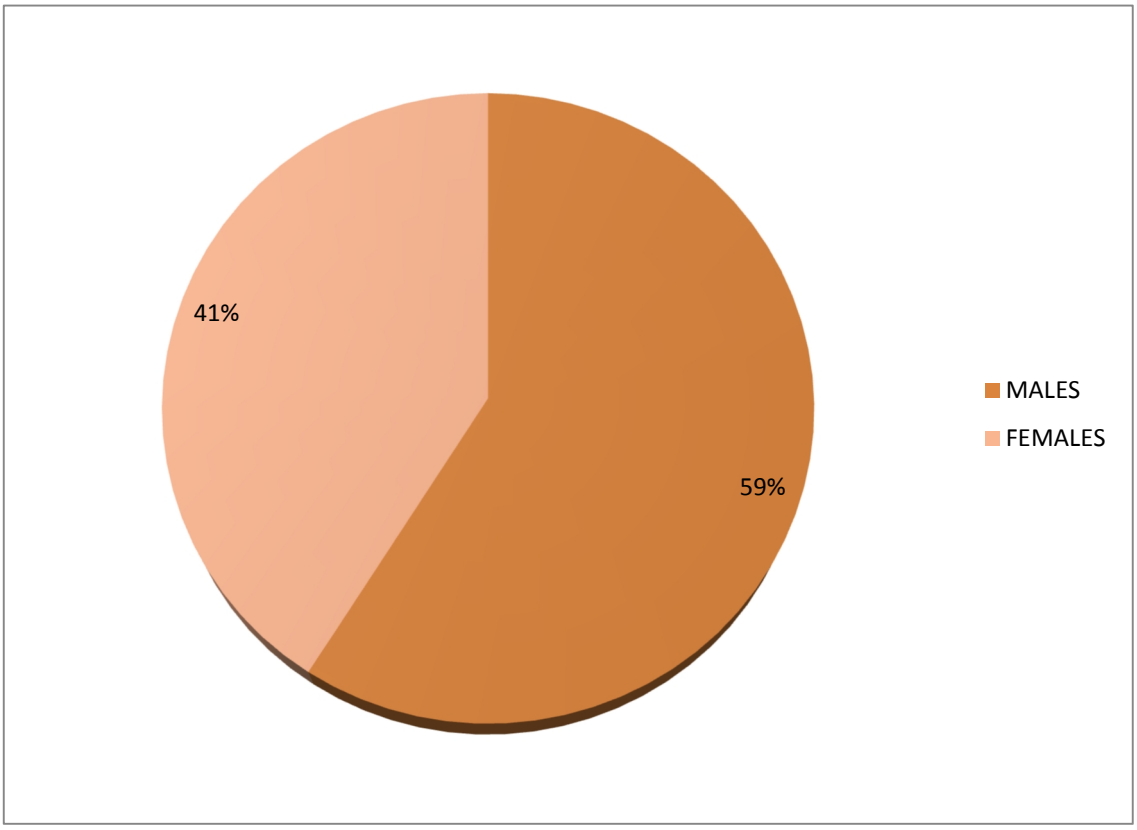

Figure 1: GENDER DISTRIBUTION

In the present study 59% of babies were males and 41% were females. The male to female ratio was 1.43 : 1 .

TABLE 2. AGE DISTRIBUTION

AGE (Days)	DISTRIBUTION(n=100)	
	NUMBER	PERCENTAGE
1-5	90	90.00
6-10	7	7.00
>10	3	3.00
TOTAL	**100**	**100.00**

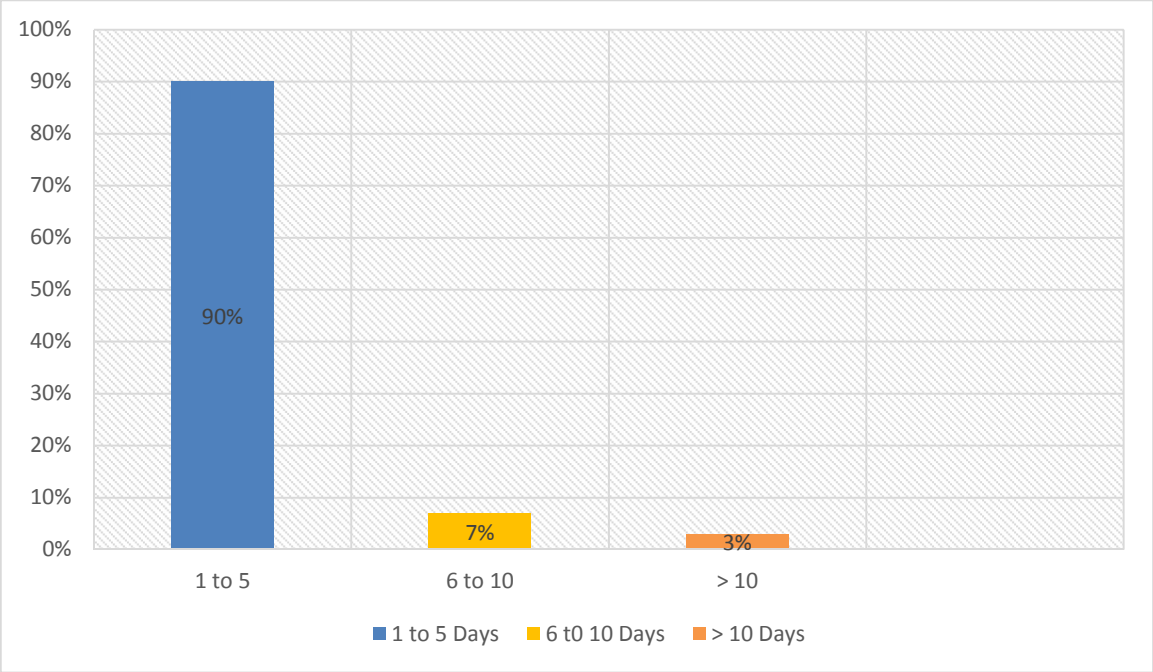

Figure 2 : AGE DISTRIBUTION

In this study the commonest age group was 1 to 5 days with 90% of neonates, followed by 6 to 10 days with 7% and more than 10 days comprised of 3% of neonates.

TABLE 3. BIRTH WEIGHT.

BIRTHWEIGHT(grams)	DISTRIBUTION (n=100)	
	NUMBER	PERCENTAGE
<1500	9	9.00
1500 to 2499	54	54.00
2500 to 3499	34	34.00
≥3500	3	3.00
TOTAL	**100**	**100.00**

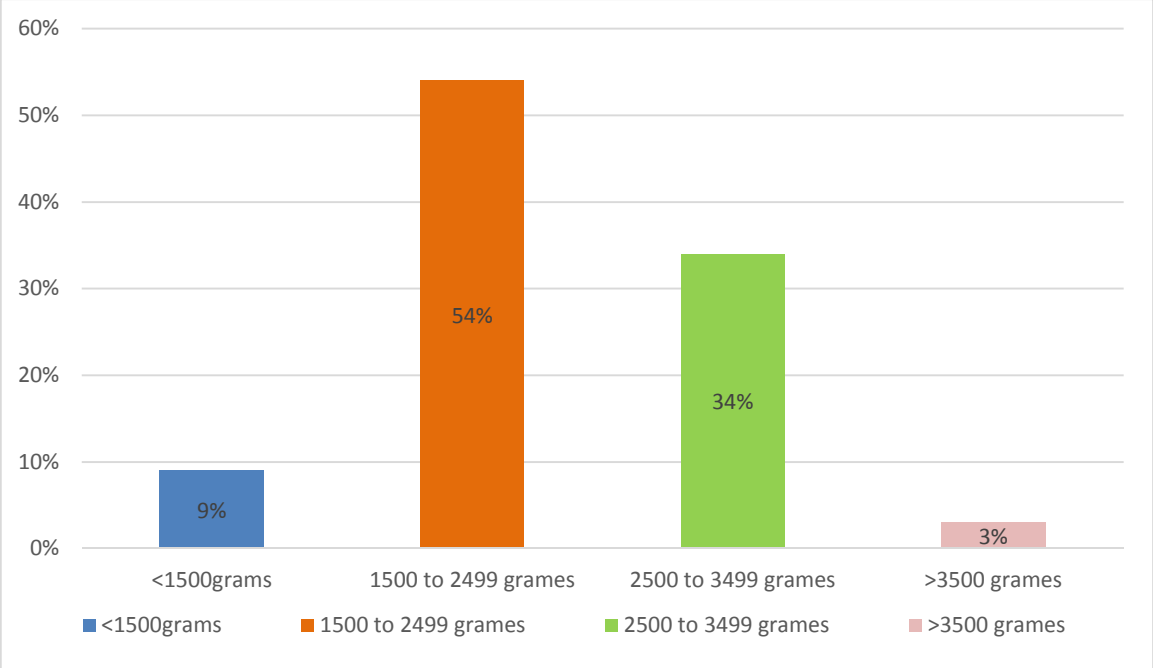

Figure 3: BIRTH WEIGHT

In the present study 54% of the neonates had birth weight between 1500 to 2499 grams and among 37% of the neonates had birth weight more than2500 grams. The mean birth weight was 2300±632 grams.

TABLE 4. MODE OF DELIVERY

MODE OF DELIVERY	DISTRIBUTION (n=100)	
	NUMBERS	PERCENTAGE
NORMAL	42	42.00
CESSARIAN	58	58.00
TOTAL	**100**	**100.00**

In the present study, 42% of mothers had normal delivery, of which, one was forceps and one was vacuum delivery. 58% of mothers had caesarian sections.

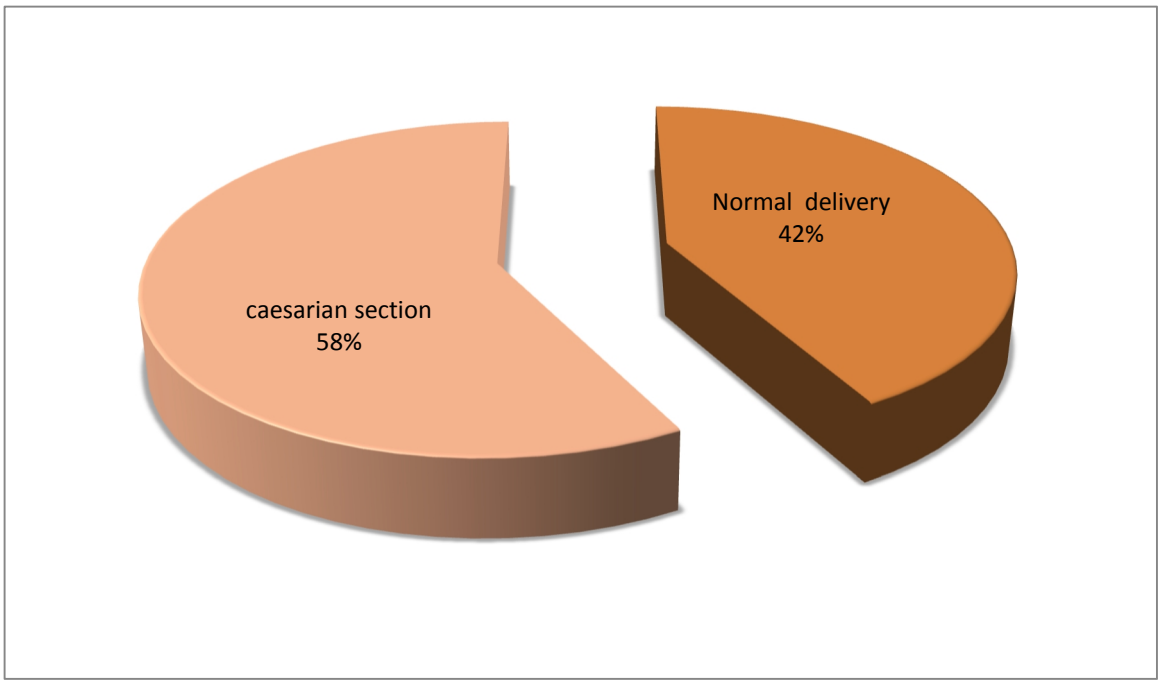

Figure 4 : MODE OF DELIVERY

TABLE 5. MATERNAL RISK FACTORS

HISTORY	NUMBER
PREMATURITY	54
PREMATURE RUPTURE OF MEMBRANES(PROM)	24
OTHER COMPLICATIONS	19

In this study of 100 neonates, maternal history included prematurity in 54 cases and premature rupture of membranes in 24 cases. These features showed overlapping in many cases.

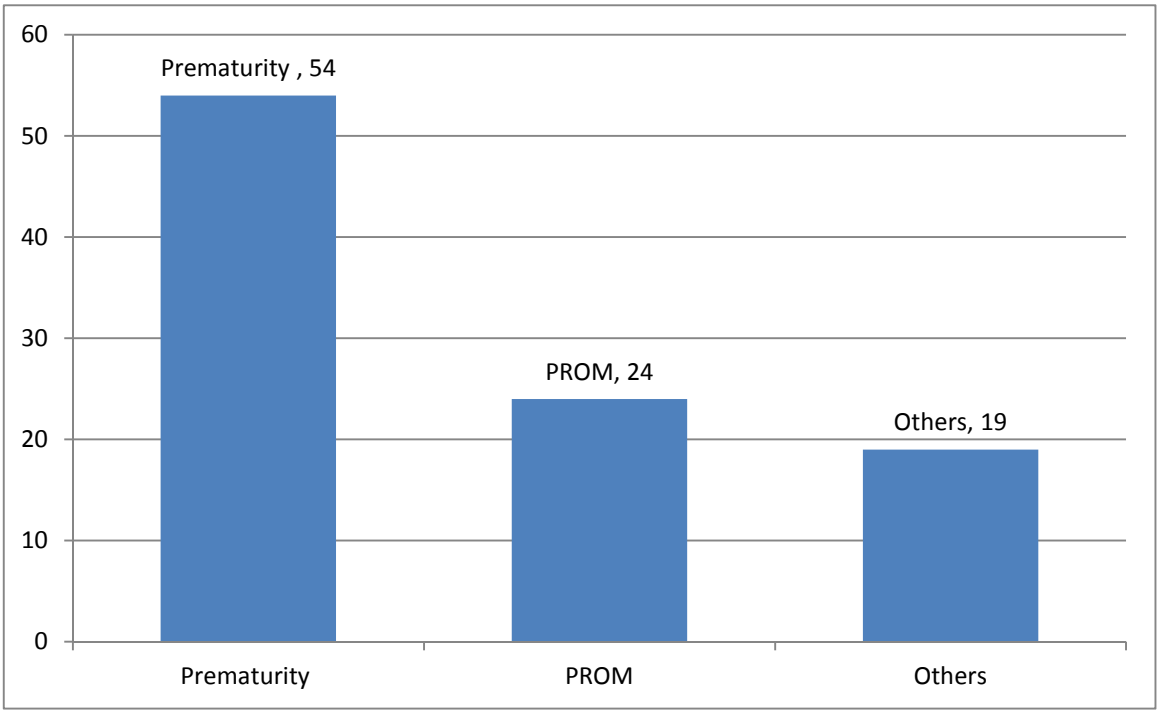

Figure 5 : MATERNAL RISK FACTOR

TABLE 6. GESTATIONAL AGE.

GESTATIONAL AGE	DISTRIBUTION (n=100)	
	NUMBER	PERCENTAGE
PRE TERM	54	54.00
TERM	46	46.00
TOTAL	**100**	**100.00**

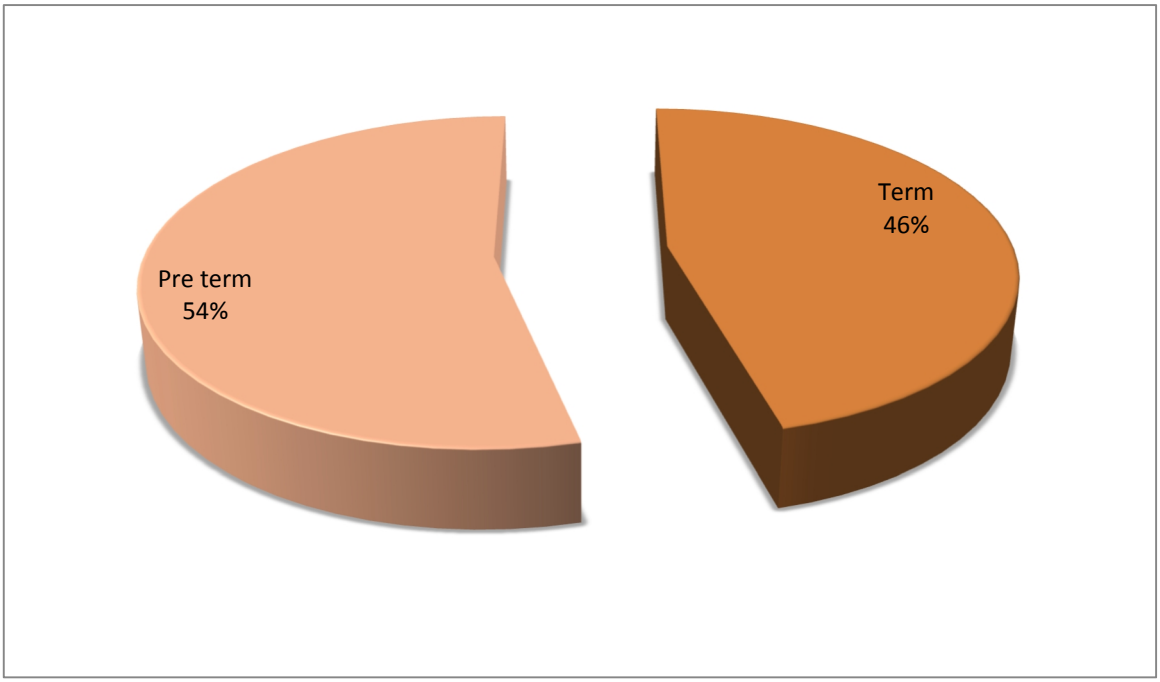

Figure 6 : GESTATIONAL AGE

TABLE 7. COMPLAINTS

COMPLAINTS	NUMBER
REFUSAL TO FEED	59
RESPIRATORY DISTRESS	32
REDUCED MOVEMENT	23
FEVER	5
JAUNDICE	1
OTHERS	8

In the present study, refusal to feed and respiratory distress were the commonest complaints present in 59 and 32 neonates respectively. The other complaints were reduced movements, fever, jaundice, seizures etc. Majority of the symptoms show overlapping.

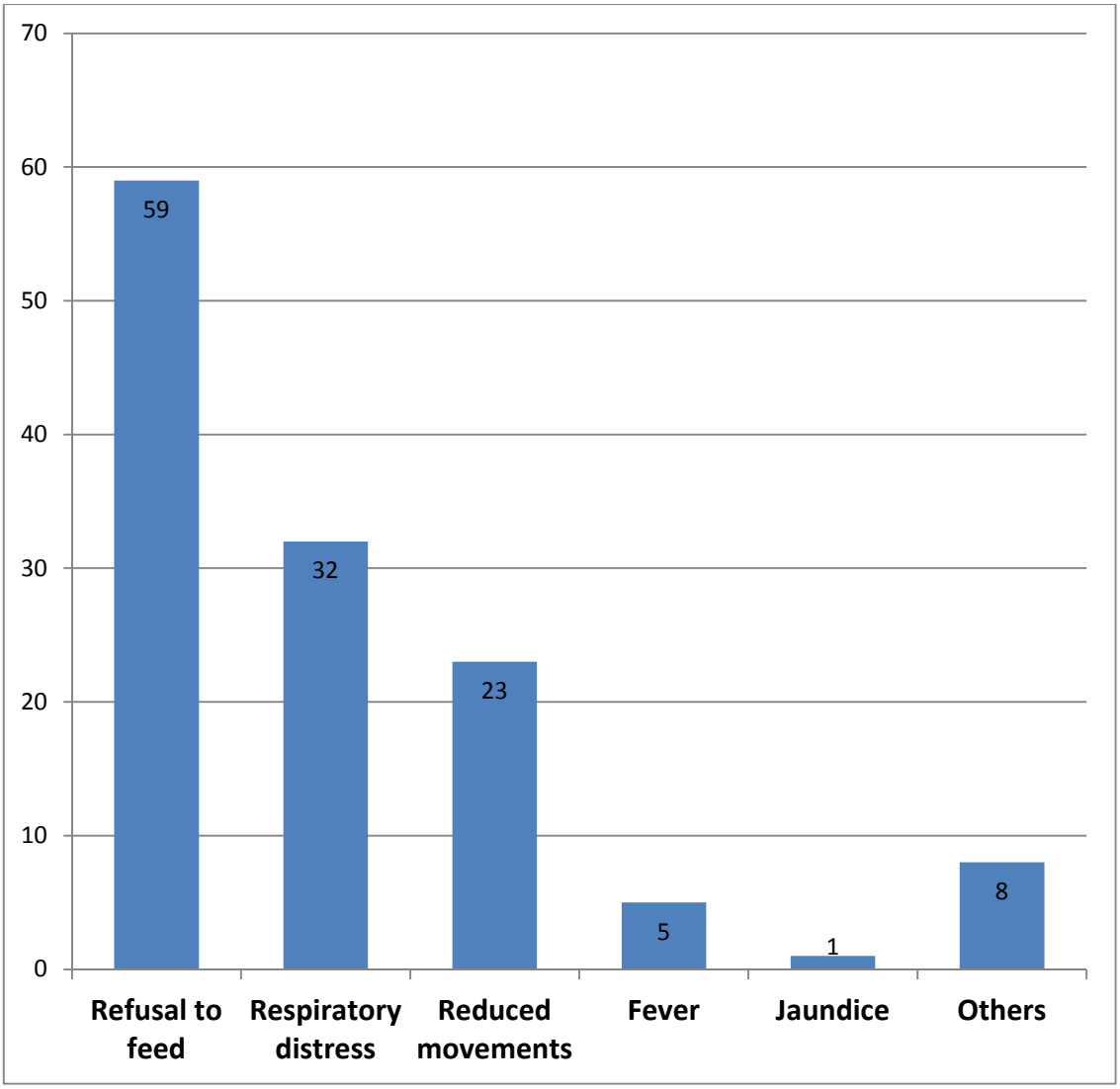

Figure 7 : COMPLAINTS

TABLE 8. PERIPHERAL SMEAR ABNORMALITIES

FINDINGS	NUMBER
Neutrophilia	92
Degenerative changes	37
Thrombocytopenia	19
Leucocytosis	14
Nucleated RBCs(>5/100WBCs)	12
Leucopenia	3

In the present study, peripheral blood flim examination revealed neutrophilia as the most common finding present in 92 out of 100 cases, followed by degenerative changes in 37 cases, thrombocytopenia in 19 cases, leukocytosis in 14 cases, nucleated red blood cells in 12 cases and leucopenia in 3 cases. In addition to this eosinopenia, neutrophil macrocytosis, neutrophil clustering were some of the hematological features noted in the peripheral smear examination of sepsis suspicious babies.

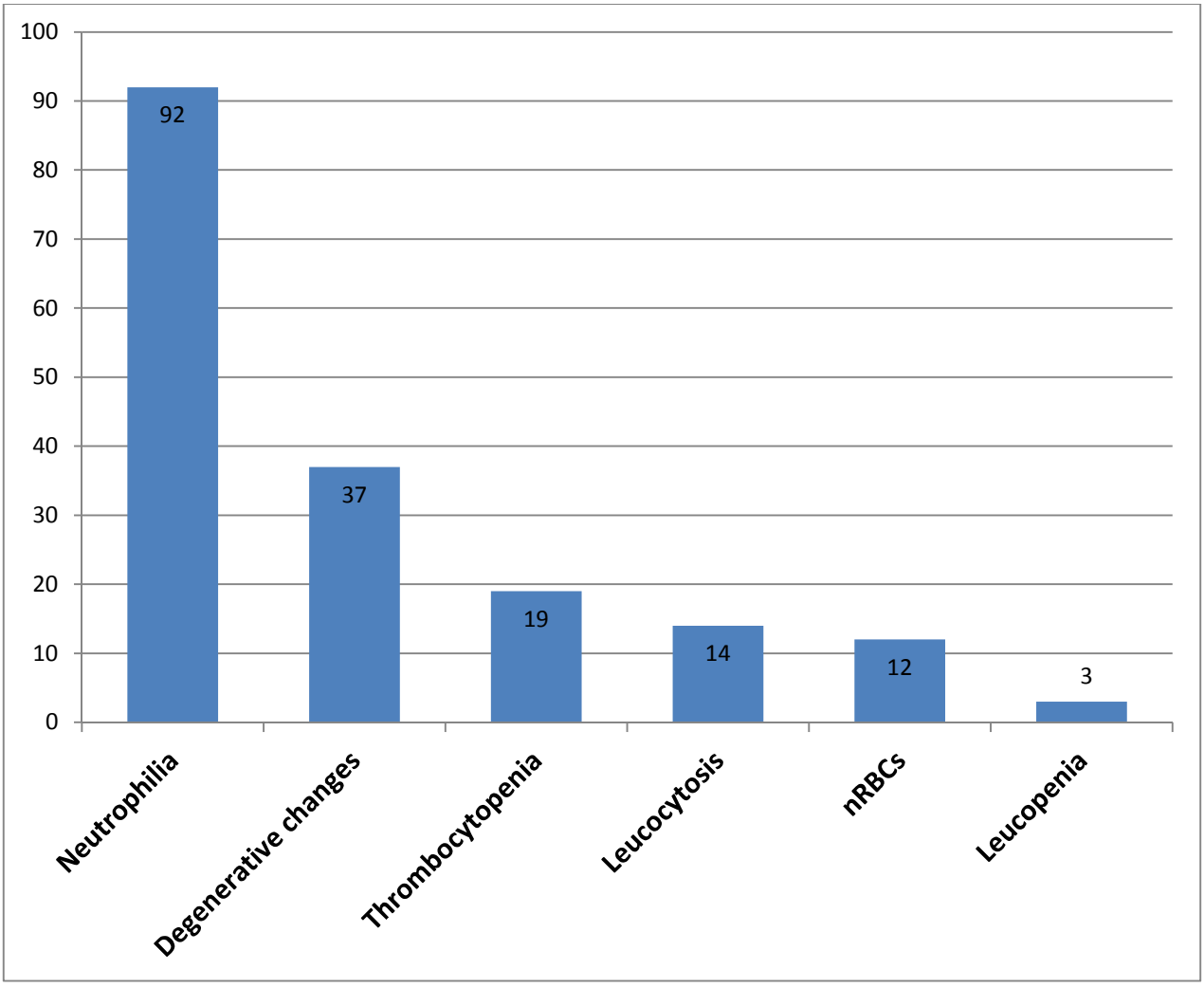

Figure 8 : PERIPHERAL SMEAR ABNORMALITTIES

TABLE 9. C- REACTIVE PROTEIN

RESULT	DISTRIBUTION (n=100)	
	NUMBER	PERCENTAGE
POSITIVE	26	26.00
NEGATIVE	74	74.00
TOTAL	**100**	**100.00**

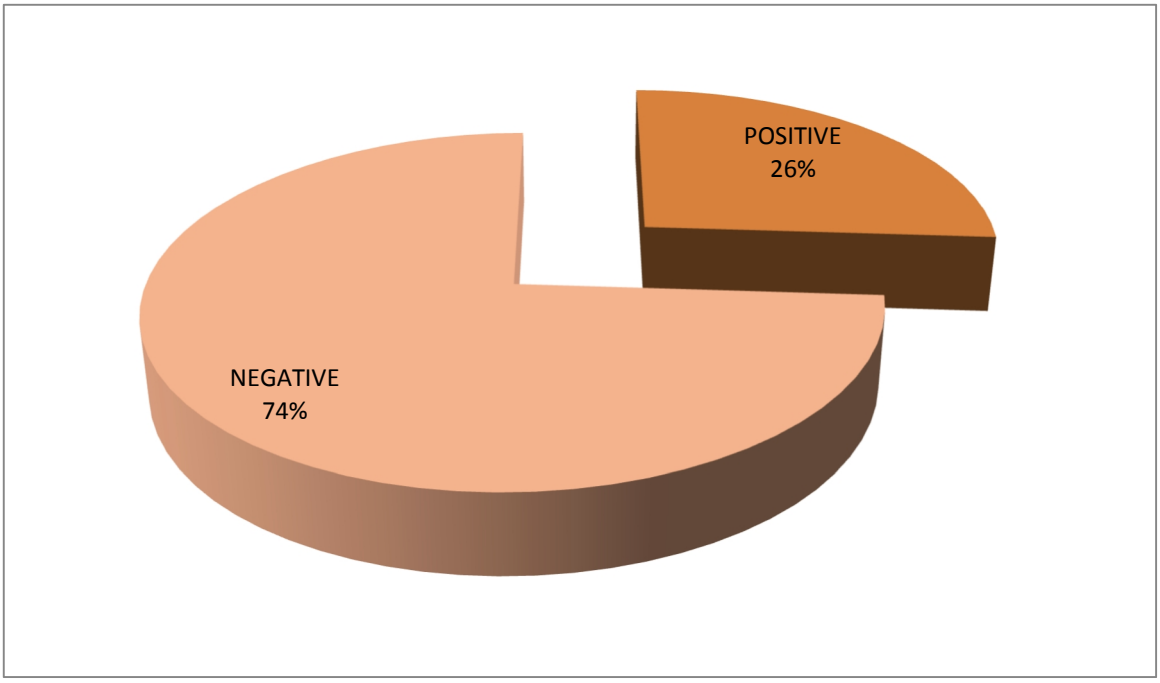

Figure 9 : C-REACTIVE PROTEIN

In this study, C-Reactive protein was positive in 26% of neonates and negative in the remaining 74%.

TABLE 10 DIAGNOSTIC ACCURACY OF C-REACTIVE PROTEIN IN PREDICTING SEPSIS

CRP	CULTURE		TOTAL
	POSITIVE	NEGATIVE	
POSITIVE	16	10	26
NEGATIVE	14	60	74
TOTAL	**30**	**70**	**100**

CRP	SENSITIVITY(%)	SPECIFICITY(%)	PPV(%)	NPV(%)
	53.3	85.7	61.5	81.1

In this present study of the 30 neonates with sepsis, 16 neonates were C-reactive protein positive and 14 were negative($p< 0.001$).The sensitivity of C-reactive protein in predicting sepsis was 53.3%, specificity was 85.7%, positive predictive value was 61.5 % and negative predictive value was 81.1%.

TABLE 11. BLOOD CULTURE

BLOOD CULTURE RESULTS	DISTRIBUTION(n=100)	
	NUMBER	PERCENTAGE
POSITIVE	30	30.00
NEGATIVE	70	70.00
TOTAL	**100**	**100.00**

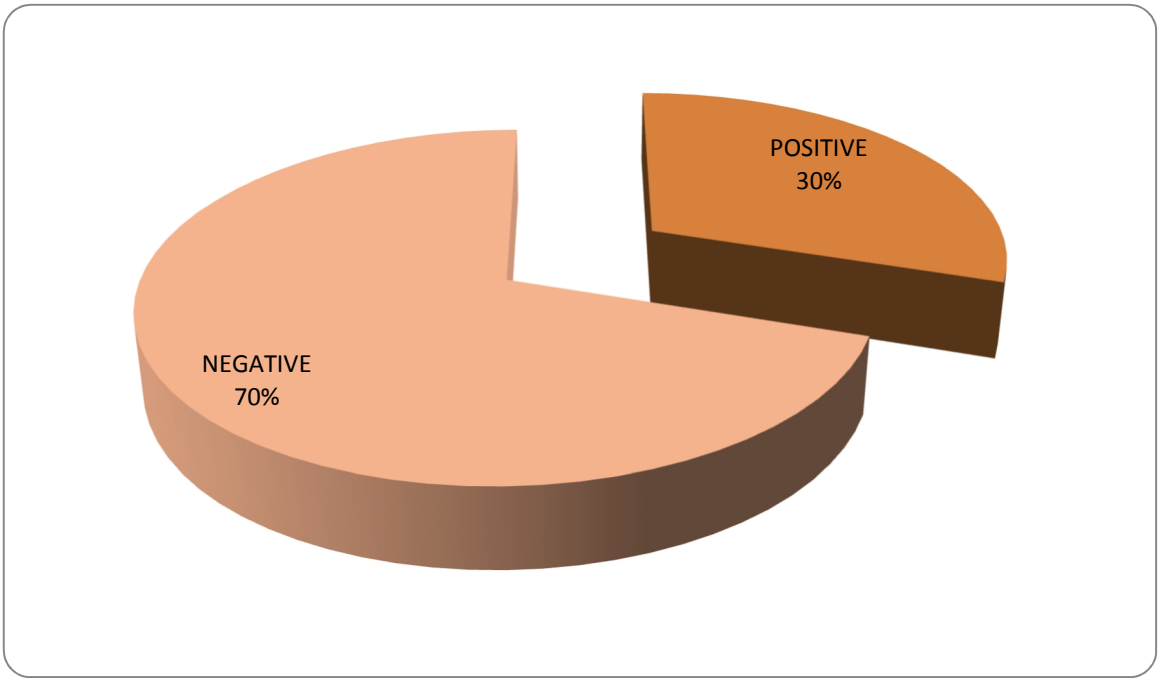

Figure 10 : BLOOD CULTURE

In this present study, the blood culture was positive in 30% of cases and negative in the remaining 70% of cases.

TABLE 12. DIAGNOSTIC ACCURACY OF HAEMATOLOGICAL SCORING SYSTEM

HAEMATOLOGICAL SCORE	CULTURE		TOTAL
	POSITIVE	NEGATIVE	
≥ 5	4	0	4
<5	26	70	96
TOTAL	**30**	**70**	**100**

SCORE	SENSITIVITY(%)	SPECIFICITY(%)	PPV(%)	NPV(%)
≥ 5	13.3	100	100	72.9

In this present study of the 30 neonates with sepsis, 4 neonates had haematological scoring system score of ≥ 5 and 26 had score of less than 5 (p= 0.002).The sensitivity of haematological scoring system with cut-off score of 5 in predicting sepsis was 13.3% and specificity was100%.

TABLE 13. DIAGNOSTIC ACCURACY OF SCORING SYSTEM

HAEMATOLOGICAL SCORE	CULTURE		TOTAL
	POSITIVE	NEGATIVE	
≥ 4	28	26	54
<4	2	44	46
TOTAL	**30**	**70**	**100**

SCORE	SENSITIVITY(%)	SPECIFICITY(%)	PPV(%)	NPV(%)
≥ 4	93.3	62.9	51.9	95.7

In this present study of the 30 neonates with sepsis, 28 neonates had haematological scoring system score of ≥ 4 and 2 had score of less than 5 ($p<0.001$).The sensitivity of haematological scoring system with cut-off score of 4 in predicting sepsis was 93.3% and specificity was 62.9%.

DISCUSSION

Neonatal sepsis is a clinical syndrome characterized by signs and symptoms of infection in the first month of life with or without bacteremia. Early recognition and diagnosis of neonatal sepsis is difficult because of its nonspecific clinical presentation(72). Aggarwal et al reported that sepsis was the commonest cause of neonatal mortality in each year in the developing countries and was responsible for 30-50% of the total neonatal deaths. The incidence of neonatal sepsis was reported to be 38 per 1000 live births in tertiary care institutions.(73) Neonatal sepsis can be early or late onset. Eighty-five percent of newborns present within 24 hours, with early onset of infection, 5% present at 24-48 hours, and a smaller percentage of patients present between 48 hours and 6 days of life. The newborns are susceptible due to immaturity of both the cellular and humoral immune systems at birth. This is very much evident in preterm neonate. The infection is transmitted from the mother through transplacental route to the fetus, ascending infection, and infection upon passage through an infected birth canal or exposure to infected blood at delivery(74). Late-onset sepsis (LOS) when infection is demonstrated in blood and cerebrospinal fluid cultures after 7 days from delivery is caused by postnatal nosocomial or community sources of the pathogen.(9)

In neonatal septicemia cases, early diagnosis is mainly based on clinical evaluation. The laboratory diagnosis of neonatal sepsis requires a microbiologic-clinical correlation. Many sepsis suspicious neonates were treated empirically with higher antibiotics for several days, while waiting for microbiological blood culture (takes 48-72hrs), and the yield of a positive blood culture is low, ranging from 8-73% as shown in various studies(3) and the facilities for the test may not be available in many laboratories.

Therefore, there is a need for a test, that is easily performed, quick, simple and cost effective with maximum sensitivity and specificity for identifying neonatal sepsis. The present study was done in order to evaluate the role of seven parameters of Rodwell's(68) hematological scoring system for the early detection of neonatal sepsis.

In this present study, 100 sick neonates admitted in Neonatal Intensive Care Unit, Tirunelveli Medical College Hospital, who were suspicious to have sepsis, based on maternal and neonatal risk factors were studied. Their peripheral smears obtained by heel prick method were evaluated according to Rodwell's seven criteria Hematological Scoring System. In this study, 30% neonates were proven to have sepsis by the gold standard blood culture method. However rest of the suspected sepsis groups (70%) makes a difficult diagnostic group and they could not be just ignored.

In this study we divided the neonates age wise in to three different groups. The commonest age group was 1 to 5 days with 90% of neonates , followed by 6 to 10 days with 7% and more than 10 days comprised of 3% of neonates. Therefore, most of the neonates presented within first week of their life. The present study group of suspected sepsis cases shows male predominance of 59%, and females constitute 41%.The male to female ratio is 1.43:1 This was in par with the study of Khalada Binte Khair (75) (males 58% and females42%) , Shukla, Aditi Rawat(76)(males 58% and females42%) . The male predominance may be due to globin synthesis regulating factor situated on the X chromosome, which makes male neonates less immunologically protected against infections.

In the present study we divide the birth weight into four categories, of which 54% of the neonates had birth weight between 1500 to 2499 grams, followed by 34% in between 2500 to 3499 grams, 9% were less than 1500 grams and 3% of neonates had more than 3500grams. The mean birth weight was 2300±632 grams. Most of the neonates (54%) were under low birth weight. Very low birth weight constitutes 9%. Similar results were seen in study of Khurshid Anwer and Sultan Mustaf,(78) where sixty-six percent neonates (33 of 50) were <2.5 kg. Another study by Lakhey, Shakya(77) reported birth weight less than 2500 gms (low birth weight) was present in 51(70.8%) culture positive cases. Low birth weight and very low birth weight babies are more susceptible to infection because of low

levels of immunoglobulins and lower defence mechanism.(7) In our study 58% of neonates were born through caesarian section compared to normal delivery which constitutes about 42% which includes one forceps and one vacuum assisted delivery. Premature rupture of membrane (less than 37weeks) in about 54% of mothers and prolonged rupture of membrane(more than 18hrs) in 24% of mothers were found to be the most common maternal high risk factors. Other risk factors such as maternal fever, foul smelling discharge per vagina, maternal urinary tract infections etc. Constitutes 19%. This was in par with the study by S.Khurshid Anwer and Sultan Mustafa(78), they said prematurity (less than 36 weeks), prolonged rupture of membranes (>24 hours) were the common maternal risk factors .Chan et al(10) newborns of mothers with risk factors for infection (defined as prelabour rupture of membranes [PROM], preterm <37 weeks PROM, and prolonged ROM) had a 2.3 (95% CI 1.0–5.4) times higher chance of infection than newborns of mothers without risk factors.

In our study, pre-term neonates were 54% and term neonates were 46%. Similar findings were observed in a study from Haryana which reported 57% pre-term babies and 43% term babies to had sepsis(79) . The higher prevalence of neonatal sepsis in pre-term babies than term babies are due to their poor immune system, low levels of immunoglobins and low birth weight at birth. In this study the chief nonatal complaints were poor feeding/refusal to feed in about 59% of cases, followed by respiratory

distress in about 32% neonates. The other complaints were reduced movements (23%), fever(5%), jaundice(1%), seizures(1%) etc.. Majority of the symptoms show overlapping. Munazza Saleem(80) on analysis of data found that history of neonatal fever was the most common presentation (79.4%) followed by poor feeding (66.5%) and depressed neonatal reflexes (54.7%). In our study poor feeding was the most common presentation followed by respiratory distress. In the present study, peripheral blood film examination revealed neutrophilia as the most common finding present in 92 out of 100 cases, followed by degenerative changes in 37 cases, thrombocytopenia in 19 cases, leukocytosis in 14 cases, nucleated red blood cells in 12 cases and leucopenia in 3 cases. A study by Tripathi and Malik revealed that, increased nucleated RBC (nRBC) count immediately after birth could be an interesting marker of early-onset neonatal sepsis (EONS) in absence of hypoxia and awaits further evaluation.(81)

In this present study, C-Reactive protein was positive in 26% of neonates and negative in the remaining 74%. This is not in par with the study results of Munazza Saleem(80) where CRP was positive in 66%. CRP is one of the most widely available; most studied, and most used laboratory tests for neonatal bacterial infection. It is well known that it provides limited sensitivity when determined during the early phases of the disease, especially at the initial presentation, but provides very high

negative predictive values and is thus useful for identifying infants unlikely to be infected or monitoring the response to treatment (82). Use of CRP in neonatal sepsis is complicated by a nonspecific rise that starts shortly after birth(83). In this present study out off 100 neonates,30 neonates(30%) were proved to have sepsis by blood culture, remaining 70 neonates (70%) were blood culture negative . Gram negative organisms were commoner than gram positive bacteria. Klebsiella pneumonia(11/30)was the commonest bacteria, followed by staphylococcus aureus(9/30), coagulase negative staphylococci(5/30) . This was similar to a study from Kolkata, 62 cases studied of which 38 had positive blood culture reports. Gram negative organisms were commoner (68.4%) than gram positive organisms(31.6%). Klebsiella pneumonia was the commonest bacteria (52%) ,followed by staphylococcus aureus(26%)(84). Off the 30 sepsis proven neonates 16 neonates were C-reactive protein positive and 14 were negative. The statistical cross tabulation values between CRP and blood culture shows a significant probability value ($p< 0.001$). The sensitivity of C-reactive protein in predicting sepsis was 53.3% , specificity was 85.7%,positive predictive value was 61.5 % and negative predictive value was 81.1%.

Rodwell et al studied 287 neonates with perinatal factors or clinical suspicion of sepsis. Hematologic findings and complete blood cell count criteria were evaluated as screening tests. A hematological scoring system

was formulated that assigned a score of 1 for each of seven parameters, abnormal leucocyte count, abnormal total neutrophil count, elevated immature neutrophil count, elevated immature to total neutrophil ratio (I:T), elevated immature to mature neutrophil ratio (I:M), decreased platelet count and degenerative changes in neutrophils. Their study revealed that, higher the score the likelihood of sepsis also was higher. The present study also evaluated the seven parameters of Rodwell's Hematological Scoring System, and the results revealed that the score of more than five when compared to the score of more than four has higher likelihood of sepsis and is well correlated with Rodwell's(68) study.

In this study, to evaluate and highlight the importance of hematological scoring system in early diagnosis of neonatal sepsis we used different cut-offs of hematological scoring system. In this study, out of 30 neonates with sepsis,4 neonates had hematological scoring system score ≥ 5 and 26 had score of <5 (p=0.002). The sensitivity of haematological scoring system with cut-off score of 5 in predicting sepsis was 13.3% and specificity was100%. Positive predictive value was 100% and negative predictive value was 72.9%.

In this study, out of 30 neonates with sepsis,28 neonates had hematological scoring system score ≥ 4 and 2 had score of <4 (p=0.001). The sensitivity of haematological scoring system with cut-off score of ≥ 4 in predicting sepsis was 93.3% and specificity was62.9%. Positive

predictive value was 51.9% and negative predictive value was 95.7%. A similar study from Dhaka revealed that most of the septicemia neonates had score ≥ 4 ,and sensitivity of 100% ,specificity of 60%,Positive predictive value of 26% and negative predictive value of 100%.The Dhaka study also reported that , in comparison with score > 3,score > 4was more sensitive (p=0.001) .(85)

Further, in the present study, to evaluate and highlight the usefulness of various parameters included in the hematological scoring system in early diagnosis of neonatal sepsis, we assessed the mean, standard deviation, probability value of individual variables which are shown in the table below.

GROUP STATISTICS

SCORE FOR	CULTURE & SENSITIVITY	NUMBER	MEAN	STD. DEVIATION	STD. ERROR MEAN	p VALUE
Total count	Positive	30	0.20	0.407	0.074	0.605
	Negative	70	0.16	0.367	0.044	
Total PMN	Positive	30	0.93	0.254	0.046	0.751
	Negative	70	0.91	0.282	0.034	
Immature PMN	Positive	30	0.97	0.183	0.033	0.199
	Negative	70	0.89	0.320	0.038	
I:T PMN	Positive	30	1.00	0.000	0.000	<0.001
	Negative	69	0.61	0.492	0.059	
I:M PMN	Positive	30	0.53	0.507	0.093	0.001
	Negative	70	0.20	0.403	0.048	
Degenerative changes	Positive	30	0.53	0.507	0.093	0.027
	Negative	70	0.30	0.462	0.055	
Platelet count	Positive	30	0.37	0.490	0.089	0.001
	Negative	70	0.10	0.302	0.036	
TOTAL SCORE	Positive	30	4.53	0.819	0.150	<0..001
	Negative	70	3.17	1.035	0.124	

In this study it was observed that, all the seven parameters show a higher mean value in the culture positive cases than the culture negative cases. Among the various parameters of hematological scoring system, raised I:T ratio had a significant p value ($p<0.001$) , followed by raised I:M ratio which had a significant p value ($p=0.001$). This was similar to the studies of Rodwell and Khair KB. An I:T ratio >0.2 suggested by Rodwell,(68) had a sensitivity of 96%.In addition , study by Khair KB(75) with I:T ratio >0.2 gives a sensitivityof 100%,specificity of 4%,positive predictive value of 13% and negative predictive value of 100%.

In addition to this**,** Narasimha and Harendrakumar evaluated 50 peripheral blood smears of neonates for neonatal sepsis using HSS of Rodwell et al. criteria. They found that an abnormal immature to total neutrophil ratio (I: T), followed by an abnormal immature to mature neutrophil ratio (I: M), were most sensitive indicators in the diagnosis of neonatal sepsis. The hematological scoring system (HSS) is a simple, quick, cost effective screening tool for early diagnosis of neonatal sepsis**.** (69)

The next best parameter after I:T ratio and I:M ratio in our study was platelet count. In this study, thrombocytopenia was seen in 19% of cases. Low platelet count of <150,000/cu.mm showed a significant p value ($p=0.001$). In a study done by K.B.Khair, Asadur Rahman(75) ,they found

thrombocytopenia in 35% cases with sensitivity of 60%, specificity 82%, PPV 31% and NPV 94%. So thrombocytopenia could be used as an early marker for sepsis but it is nonspecific. Neonates with sepsis develop thrombocytopenia, possibly because of disseminated intravascular coagulation (DIC) and the damaging effects of endotoxin on platelets.

In this present study, parameters such as, total WBC count, total PMN count, I:M PMNs had insignificant p values. Similar inferences were given by Dulay et al, who studied laboratory criteria based on modification of the criteria of Rodwell et al. He found that WBC count and absolute neutrophil count (ANC) were not significant. In contrast, the associations with absolute band count, and I/T neutrophil ratio continued to remain significant.(86) In our study, degenerative changes in neutrophils such toxic granules ,nuclear or cytoplasmic vaculation were seen in 37% of sepsis suspicious cases.

Cheng-HurdLui et al reviewed 195 peripheral blood smears of 157 neonates who required sepsis work up and found that vacuolization and toxic granulation of neutrophils were often found in blood smears of neonates with bacterial infection. Presence of toxic vacuolization in neutrophils were found to be more specific. Persistence of vacuolization may reflect either inadequacy of antibiotic therapy or persisting focus of infection(87) .

We evaluated various hematological parameters in early diagnosis of neonatal sepsis and found that, no single individual test is superior to other. Philip and Hewitt analysed 376 neonates during the first week of birth. They used a panel of screening tests such as, white blood cell count, band/total neutrophil ratio, C-reactive protein, micro-ESR and haptoglobin to identify the sepsis babies. The result reveals that 93% of the infants proven to have sepsis had two or more abnormal tests.(88),(89)

Since, no single individual hematological parameter is superior compared to another in predicting neonatal sepsis, the combination of all the above parameters in the form of HSS has been recommended. The HSS can be performed in all infants, including those who have received antibiotic therapy prior to evaluation and simplifies the interpretation of hematologic profile.

SCORE	SENSITIVITY (%)	SPECIFICITY (%)	PPV (%)	NPV (%)	p VALUE
≥4	93.3	62.9	51.9	95.7	<0.001
≥5	13.3	100	100	72.9	0.002

In this study, with the cut-off HSS score as ≥4, sensitivity was 93.3%, specificity was 62.9%, positive predictive value was 51.9%, negative predictive value was 95.7%. When the cut-off HSS score was ≥5 the sensitivity was 13.3%, specificity was 100%, positive predictive value was 100% and negative predictive value was 72.9%. In comparison with score ≥5, score ≥4 was more sensitive (P <0.001). Specificity and PPV were significantly higher as well, 62.9% and 51.9% respectively. But considering the high specificity, positive predictive value this study implies that score ≥ 4 was more reliable as a screening tool for sepsis than any of the individual hematological parameter. Ghosh et al studied the hematologic profiles of 103 high risk newborns according to the scoring system of Rodwell et al for early detection of sepsis. They found it to be a simple, quick and cost effective tool which could provide a guideline to decision making regarding antibiotic therapy.

SUMMARY AND CONCLUSION

Neonatal sepsis is a life-threatening condition, yet mortality can be very much reduced when detected early. The hematological scoring system is a very useful test to distinguish the infected from non-infected infants. The present study showed,

- The male to female ratio was 1.43:1. The commonest age group was 1 to 5 days and 63% were low birth weight. The mean birth weight of the study population was 2300 ± 632 grams.
- Of the 100 neonates 54 were preterm babies. Refusal to feed and respiratory distress were the commonest complaints present in 59 and 32 neonates respectively.
- Blood culture positive sepsis was seen in 30% of cases. Gram negative organisms were commoner than gram positive bacteria and Klebsiella pneumonia (11/30) was the commonest bacteria which caused sepsis.
- Looking into the indivudial parameters of HSS, all the seven hematological parameters show higher mean value in the culture positive cases . Raised I:T neutrophil ratio had a significant p value ($p<0.001$) , raised I:M neutrophil ratio had a significant p value ($p=0.001$), platelet count with p value($p= 0.001$). Degenerative changes in neutrophils, I:M PMNs, total WBC count, total PMN counts did not show significant p value.

- HSS with a cut-off score of ≥ 4 diagnosed 28 out of 30 culture positive cases which yielded a sensitivity of 93.3%, specificity of 62.9%, Positive predictive value of 51.9% and negative predictive value of 95.7%.
- Elevated nucleated red blood cell, decreased in eosinophil count (eosinopenia), neutrophil macrocytosis, neutrophil clustering were some of the hematological features noted in the peripheral smear examination of sepsis babies in our study, which needs further studies and to be evaluated.

We concluded that the hematologic scoring system is a useful test to distinguish the infected from non-infected infants. The hematologic scoring system is a simple, quick, cost effective and readily available tool with high sensitivity and specificity in the early diagnosis of neonatal sepsis. So it can be very well used as a screening test for early diagnosis of neonatal sepsis.

In our study HSS with a cut-off score of 4 may provide a guideline to the clinicians to make decisions regarding judicious use of antibiotic therapy which will be lifesaving, provide early cure, reduced mortality, shorten the hospital stay, and as well as will minimize the risk of emergence of resistant organism due to improper use of antibiotics. Thus unnecessary exposure of the neonates to antibiotic therapy can be avoided.

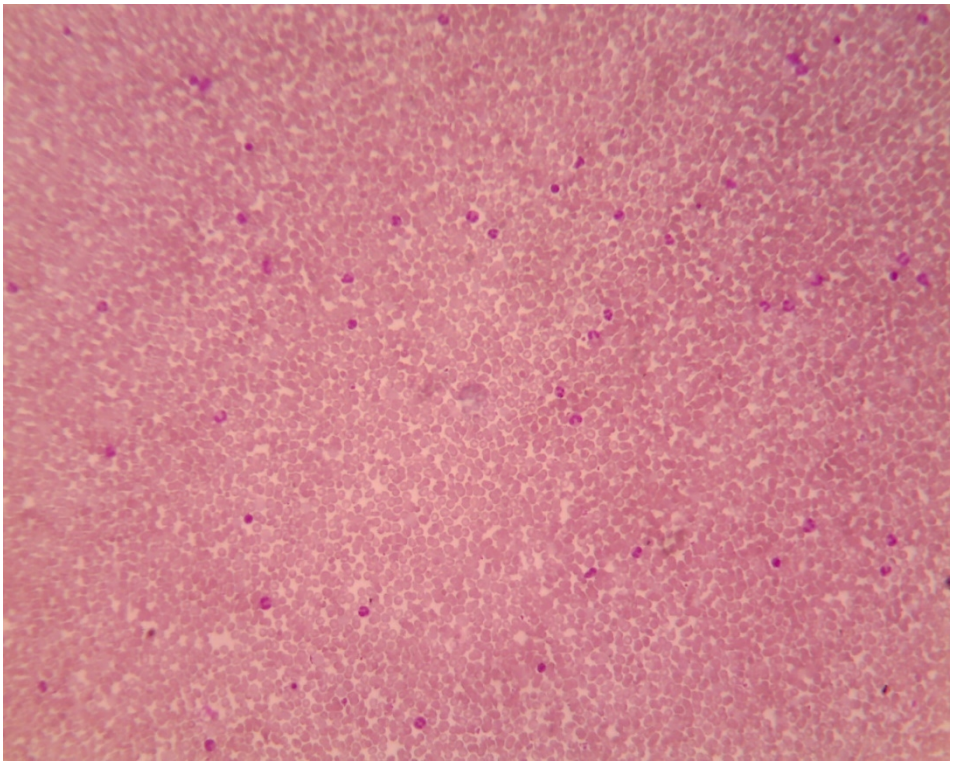

Fig i) Microphotograph of peripheral smear, Leucocytosis (Leishman stain 40X)

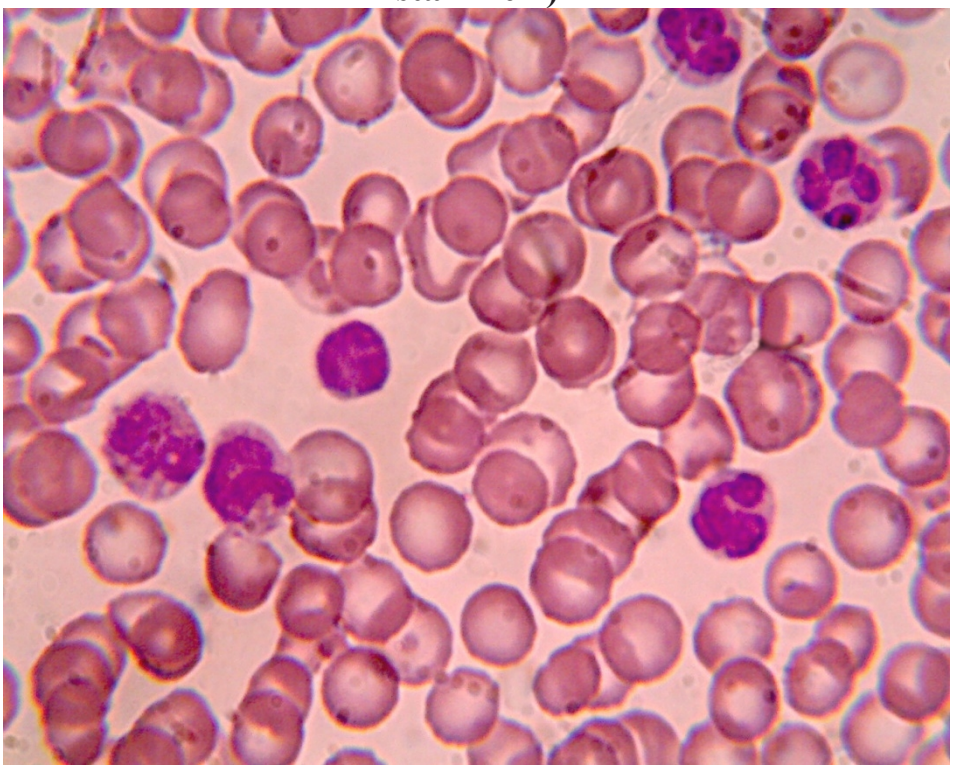

Fig ii) Microphotograph of peripheral smear, Leucocytosis(Leishman stain 1000X)

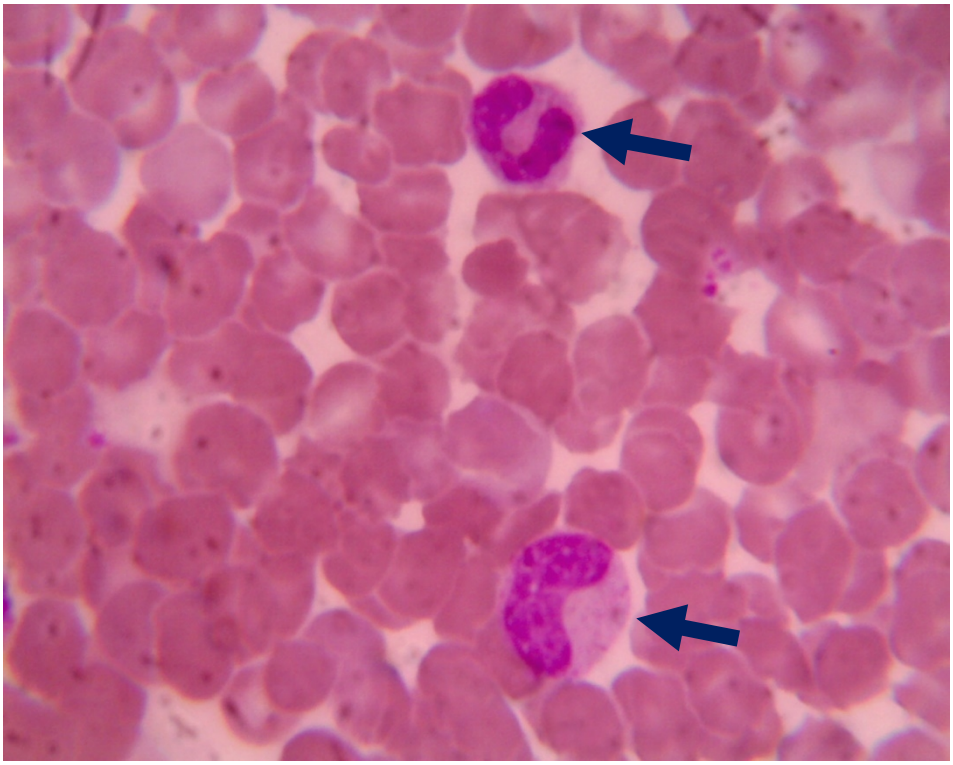

Fig iii) Microphotograph of peripheral smear, Band/Stabcells(Leishman stain 1000X)

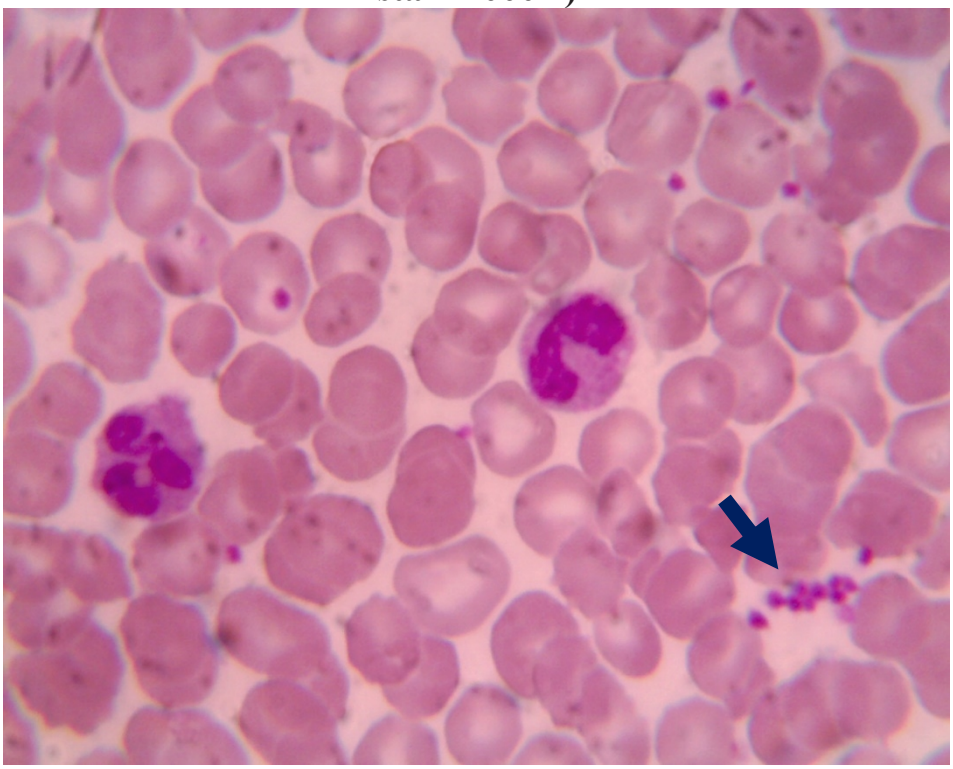

Fig iv) Microphotograph of peripheral smear, Platelet clumps (Leishman stain 1000X)

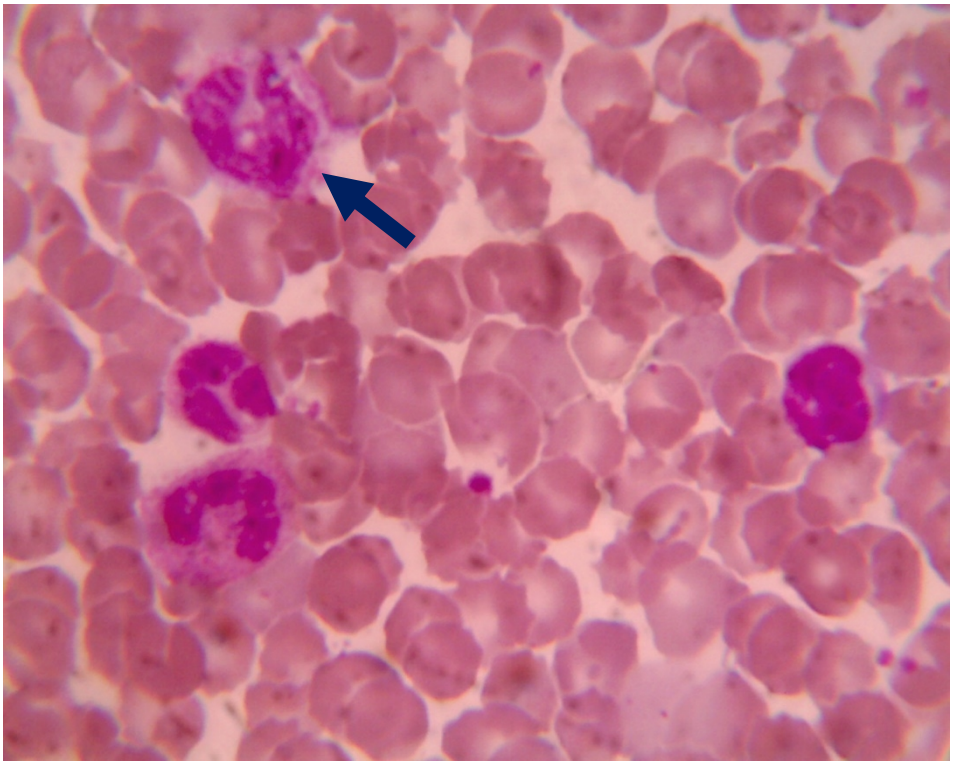

Fig v) Microphotograph of peripheral smear, Degenerative changes (Leishman stain 1000X)

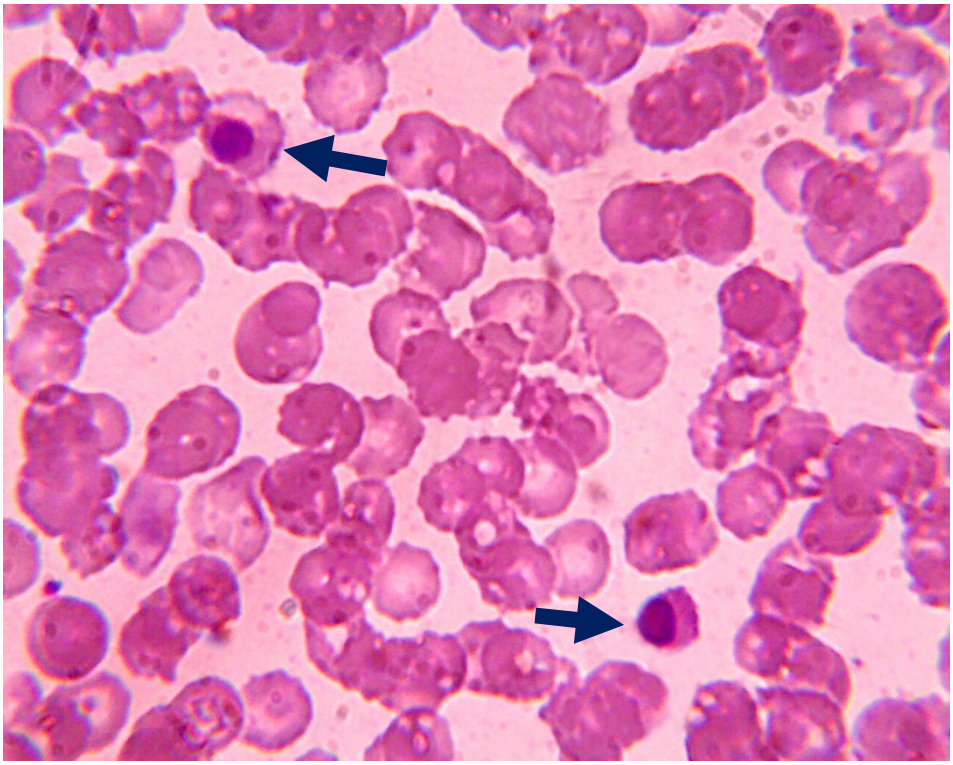

Fig vi) Microphotograph of peripheral smear, nRBCs (Leishman stain 1000X)

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CASE PROFORMA-NEONATAL SEPSIS

Name
Age
Gender
IP No
D.O.B
Time & date of admission
Chief complains
Present illness
Admitting diagnosis

Neonatal history: Gestational age,
Term/Preterm,
Birth weight

Maternal Obstetrical History:
Obstetric code,
LMP& EDD,
AN Checkups,
High risk pregnancy

Intra partum history :
Fever,
PROM,
UTI,
Foul smelling discharge PV,
Prolonged labour,
Mode of delivery,
IV Antibiotics

Past History :
Prior neonatal death,

Physical Examination:
Fever, Tachycardia, Tachypnea, irregular respiration, grunting, lethargy, poor feeding etc.

Neurological Sings:
Stupor, irritability, coma, seizures, bulging AF, Extensor rigidity, decreased tone, hypothermia etc.

Sepsis Evaluation:
Complete Blood Count
Peripheral smear examination
Blood culture
CRP value

நோயாளிகளுக்கு அறிவிப்பு மற்றும் ஒப்புதல் படிவம்
(மருத்துவ ஆய்வில் பங்கேற்பதற்கு)

ஆய்வு செய்யப்படும் தலைப்பு:
பங்கு பெறுவரின் பெயர்:
பங்கு பெறுவரின் வயது:

		பங்கு பெறுவர் இதனை ✓ குறிக்கவும்
1.	நான் மேலே குறிப்பிட்டுள்ள மருத்துவ ஆய்வின் விவரங்களை படித்து புரிந்து கொண்டேன். என்னுடைய சந்தேகங்களை கேட்கவும், அதற்கான தகுந்த விளக்கங்களை பெறவும் வாய்ப்பளிக்கப்பட்டுள்ளது என அறிந்து கொண்டேன்.	☐
2.	நான் இவ்வாய்வில் தன்னிச்சையாக தான் பங்கேற்கிறேன். எந்த காரணத்தினாலோ எந்த கட்டத்திலும், எந்த சட்ட சிக்கலுக்கும் உட்படாமல் நான் இவ்வாய்வில் இருந்து விலகி கொள்ளலாம் என்றும் அறிந்து கொண்டேன்.	☐
3.	இந்த ஆய்வு சம்பந்தமாகவோ, இதை சார்ந்து மேலும் ஆய்வு மேற்காள்ளும் போதும் இந்த ஆய்வில் பங்குபெறும் மருத்துவர் என்னுடைய மருத்துவ அறிக்கைகளை பார்ப்பதற்கு என் அனுமதி தேவையில்லை என அறிந்து கொள்கிறேன். நான் ஆய்வில் இருந்து விலகிக் கொண்டாலும் இது பொருந்தும் என அறிகிறேன்.	☐
4.	இந்த ஆய்வின் மூலம் கிடைக்கும் தகவலையோ, முடிவையோ பயன்படுத்திக் கொள்ள மறுக்க மாட்டேன்.	☐
5.	இந்த ஆய்வில் பங்கு கொள்ள ஒப்புக் கொள்கிறேன் எனக்கு கொடுக்கப்பட்டஅறிவுரைகளின் படி நடந்து கொள்வதுடன், ஆய்வை மேற்கொள்ளும் மருத்துவ அணிக்கு உண்மையுடன் இருப்பேன் என்று உறுதியளிக்கிறேன். என் உடல் நலம் பாதிக்கப்பட்டாலோ, அல்லது எதிர்பாராத, வழக்கத்திற்கு மாறான நோய்குறி தென்பட்டாலோ உடனே இதை மருத்துவ அணியிடம் தெரிவிப்பேன் என உறுதி அளிக்றேன்.	☐

பங்கேற்பவரின் கையொப்பம் / ..... இடம் .....
கட்டைவிரல் ரேகை
பங்கேற்பவரின் பெயர் மற்றும் விலாசம் .....
ஆய்வாளரின் கையொப்பம் /..... இடம் .....
ஆய்வாளரின் பெயர் .....
மையம் .....
கல்வியறிவு இல்லாதவற்கு (கைரேகை வைத்தவர்களுக்கு) இது அவசியம் தேவை
சாட்சியின் கையொப்பம் /..... இடம் .....
பெயர் மற்றும் விலாசம் ....

S.NO	IP number	Mode 0f Delivery	Gestational age	Sex	Weight in kg	Day	Risk factors	Clinical features	Total count µL	score	Total PMN	score	Immature PMN	score	I:T PMN ratio	score	I:M ratio	score	Changes in PMN	Score	Platelet count	Score	Total score	CRP	C/S	NRBCs
1	54108/17	N	T	M	3	1	PROM	poor sucking	23800	0	19873	1	4760	1	0.23	1	0.3	1	N0	0	2.4	0	4	negative	negative	0
2	54232/17	C	T	M	3.4	1	PROM	Lathargic/Grunt	16000	0	8880	1	1120	1	0.12	1	0.14	0	yes	1	2.5	0	4	negative	negative	4
3	54312/17	N	T	F	2.2	2	LBW	poor sucking	15500	0	13800	1	2100	1	0.15	1	0.17	0	Yes	1	1.2	1	5	negative	Positive	0
4	54329/17	C	P	F	2	1	PT/LBW	pink,alert	21200	0	16536	1	3180	1	0.19	1	0.23	0	N0	0	3.3	0	3	negative	negative	1
5	54386/17	N	P	F	1.9	1	PT/LBW	pink,alert	12800	0	11456	1	960	1	0.08	0	0.09	0	yes	1	1.6	0	3	negative	negative	0
6	54412/17	N	P	F	2.3	3	PT/LBW	poor sucking	19300	0	12159	1	2798	1	0.23	1	0.29	0	yes	1	2.7	0	4	positive	Positive	0
7	54492/17	C	P	M	2	7	PT/LBW	Lathargic/Grunt/PS	2400	1	1716	1	348	0	0.2	1	0.25	0	yes	1	0.31	1	5	positive	Positive	0
8	54683/17	N	T	M	2.5	1	Fever	pink,alert	20000	0	14400	1	2100	1	0.14	1	0.17	0	no	0	3.7	0	3	negative	negative	0
9	55150/17	N	T	F	2.6	2		pink,alert	18000	0	16110	1	3510	1	0.21	1	0.28	0	no	0	4	0	3	negative	negative	0
10	55821/17	C	T	M	3	1	PROM	Lathargic/Grunt	22500	0	9900	1	2587	1	0.26	1	0.35	1	yes	1	4.5	0	5	negative	negative	0
11	56101/17	C	P	M	2	3	PT/LBW	poor sucking	10000	0	7600	1	1950	1	0.25	1	0.34	1	yes	1	2.5	0	5	negative	negative	0
12	57324/17	C	T	M	3.7	1	PROM	Lathargic/Grunt	23300	0	16776	1	4427	1	0.26	1	0.32	1	yes	1	5.5	0	5	positive	Positive	0
13	57830/17	N	P	F	1.9	8	PT/LBW	poor sucking/ICR	6200	0	4278	0	1488	1	0.3	1	0.5	1	n0	0	1.4	1	4	positive	Positive	4
14	68234/17	C	P	M	1.1	1	PT/VLBW/PROM	Lathargic/Grunt/PS	16000	0	10000	1	3840	1	0.38	1	0.62	1	yes	1	2.5	0	5	negative	Positive	1
15	68490/17	N	T	F	2.5	3		Failure to thrive	15400	0	11165	1	1848	1	0.16	1	0.19	0	NO	0	2.7	0	3	negative	negative	0
16	70524/17	C	P	F	1.6	2	PT/LBW	pink,alert	5700	0	3819	0	598	0	0.15	1	0.18	0	NO	0	2.5	0	1	negative	negative	6
17	70927/17	C	P	F	1.5	1	PT/VLBW	poor sucking	13300	0	10839	1	3059	1	0.28	1	0.36	1	NO	0	1.9	0	4	negative	negative	0
18	78652/17	C	P	M	2	1	PT/LBW/PROM	Lathargic/Grunt	12300	0	7134	1	676	1	0.09	0	0.1	0	NO	0	1.5	1	3	negative	negative	1
19	79754/17	C	P	F	2.2	1	PT/LBW/PROM	poor sucking	19900	0	15621	1	3781	1	0.24	1	0.31	1	NO	0	2.6	0	4	negative	negative	1
20	80100/17	C	T	F	2.5	13		pink,alert	26800	1	16348	1	1474	1	0.09	0	0.09	0	NO	0	5	0	3	positive	negative	2
21	82315/17	C	T	M	2.8	3		PS/ICR	21700	0	17577	1	4231	1	0.24	1	0.31	1	NO	0	2.5	0	4	positive	Positive	2
22	83523/17	C	T	M	2.8	3	Fever	ICR+	14500	0	10295	1	1957	1	0.19	1	0.23	0	NO	0	2.5	0	3	positive	negative	0
23	87976/17	N	P	M	2	1	PT/LBW	poor sucking	19000	0	15105	1	1615	1	0.1	0	0.11	0	NO	0	3.6	0	2	negative	negative	1
24	89520/17	N	T	M	2.5	2		PS/ICR	20000	0	12700	1	2900	1	0.22	1	0.3	1	yes	1	2.5	0	5	positive	Positive	0
25	89811/17	N	P	M	2.2	2	PT/LBW	Lathargic/Grunt	17400	0	9918	1	957	1	0.09	0	0.1	0	YES	1	1.8	0	3	positive	negative	0
26	90123/17	C	P	F	2.9	1	PT/PROM	poor sucking/Grunt	27600	1	12558	1	4692	1	0.37	1	0.59	1	NO	0	2.8	0	5	positive	Positive	0
27	91344/17	C	P	F	2.8	15	PT/PROM	Lathargic/Grunt	23100	0	9817	1	1386	1	0.14	1	0.16	0	YES	1	4.4	0	4	positive	negative	0
28	92355/17	C	T	M	2.8	3		pink,alert	8500	0	3357	0	722	1	0.21	1	0.27	0	yes	1	1.4	0	3	positive	negative	2
29	93180/17	C	P	M	2.4	6	PT/LBW/PROM	ICR+/PS	9800	0	6321	1	1323	1	0.2	1	0.26	0	yes	1	2.3	0	4	positive	Positive	0

30	94538/17	N	P	M	1.3	1	PT/LBW	Lathargic/PS	8800	0	2684	0	1100	1	0.4	1	0.69	1	NO	0	1.1	1	4	positive	Positive	0
31	95678/17	C	P	F	1.4	1	PT/VLBW	poor sucking	8500	0	3910	0	255	0	0.06	0	0.06	0	NO	0	1.5	0	0	negative	negative	2
32	96578/17	C	T	M	3.2	3	PROM	pink,alert	18500	0	14245	1	9942	1	0.1	0	0.14	0	YES	1	1.8	0	3	positive	negative	0
33	97839/17	N	P	M	1.5	1	PT/VLBW	Lathargic/Grunt	10700	0	7169	1	963	1	0.13	1	0.15	0	NO	0	1.1	1	4	negative	negative	3
34	97878/17	C	T	M	2	1	LBW	poor sucking	14500	0	9715	1	3117	1	0.32	1	0.47	1	NO	0	2.1	0	4	negative	negative	0
35	98761/17	C	T	F	2.4	3	LBW	poor sucking	22700	0	14755	1	3518	1	0.23	1	0.31	1	NO	0	2.2	0	4	negative	Positive	0
36	99201/17	N	P	M	2.2	3	PT/LBW/PROM	ICR+	13200	0	10428	1	1716	1	0.16	1	0.19	1	NO	0	1.7	0	4	negative	Positive	0
37	10287/18	N	P	M	2.1	1	PT/LBW	pink,alert	19300	0	8009	1	868	1	0.1	0	0.12	0	NO	0	2.1	0	2	negative	negative	0
38	12309/18	N	T	F	1.8	3	LBW	poor sucking/ICR	19400	0	14647	1	3395	1	0.23	1	0.3	1	NO	0	2	0	4	positive	Positive	0
39	13459/18	C	P	M	1.8	1	PT/LBW	Lathargic/Grunt	11200	0	6328	1	896	1	0.14	1	0.16	0	NO	0	1	1	4	negative	Positive	3
40	14902/18	N	T	M	2.2	3	LBW	poor sucking	18700	0	15427	1	2524	1	0.16	1	0.19	0	NO	0	1.7	0	3	negative	negative	0
41	15678/18	N	P	M	2.3	3	PT/LBW	PS/ICR	10000	0	6600	1	1200	1	0.18	1	0.2	0	yes	1	1.7	0	4	negative	negative	0
42	16542/18	C	P	M	0.9	1	PT/ELBW/PPROM	ICR+	17200	0	12384	1	1376	1	0.11	0	0.12	0	NO	0	1.7	0	2	positive	negative	0
43	17890/18	C	T	M	2.7	7	Breech	ICR+	17300	0	13148	1	1730	0	0.13	1	0.15	0	YES	1	3.2	0	3	negative	negative	1
44	17895/18	N	P	M	1.6	2	PT/LBW	pink,alert	13200	0	10626	1	1122	1	0.1	0	0.11	0	NO	0	2.7	0	2	negative	negative	0
45	17901/18	C	P	M	1.3	1	PT/LBW	Lathargic/Grunt	35000	1	26725	1	5075	1	1.9	1	0.2	0	NO	0	2.5	0	4	negative	negative	2
46	17910/18	C	P	F	1.7	3	PT/LBW	poor sucking/Grunt	18000	0	12600	1	4230	1	0.33	1	0.5	1	YES	1	1	1	6	negative	Positive	0
47	17918/18	N	T	M	2.8	3		ICR+/PS	12400	0	9238	1	2480	1	0.26	1	0.36	1	yes	1	2.2	0	5	negative	negative	1
48	17924/18	C	T	M	3.5	3	Breech p	ICR+	25700	1	21973	1	2527	1	0.12	1	0.14	0	NO	0	2.3	0	4	negative	Positive	0
49	17933/18	N	T	F	1.7	3	LBW	pink,alert	21400	0	19046	1	2033	1	0.1	0	0.11	0	NO	0	1.1	1	3	negative	negative	10
50	18123/18	C	P	M	2.6	1	PT/PROM	Poor sucking/fever	12000	0	5100	1	1200	1	0.23	1	0.3	1	NO	0	2.5	0	4	negative	negative	0
51	18128/18	N	T	F	2.8	3	Fever	Lathargic/Grunt	3900	1	2730	0	292	0	0.1	0	0.12	0	NO	0	2	0	1	negative	negative	0
52	18200/18	C	P	M	2.4	7	PT/LBW/PROM	poor sucking	4200	1	2499	0	399	0	0.15	1	0.19	0	NO	0	2	0	2	positive	negative	0
53	18223/18	C	T	F	2.3	3	LBW/PROM	poor sucking	16400	0	9922	1	1394	1	0.14	1	0.16	0	NO	0	0.31	1	4	positive	Positive	6
54	18229/18	C	T	F	2.4	2	LBW/PROM	poor sucking	20000	0	11400	1	3200	1	0.28	1	0.39	1	YES	1	3	0	5	negative	negative	1
55	18247/18	C	T	F	2.5	2	Failed induction	PS/ICR	17200	0	13158	1	1892	1	0.14	1	0.16	0	NO	0	1.4	1	4	negative	negative	4
56	18254/18	/Forcep	T	M	2.8	1	Forceps	pink,alert	24000	0	16920	1	3840	1	0.22	1	0.26	0	NO	0	2.5	0	3	negative	negative	6
57	18278/18	C	T	M	3	1		PS/ICR	30000	1	18900	1	1200	1	0.06	1	0.06	1	NO	0	3.5	0	5	negative	negative	4
58	18293/18	C	T	M	3.2	1	PROM	Lathargic/Grunt	15400	0	10703	1	1078	1	0.1	0	0.11	0	NO	0	3.3	0	2	negative	negative	5
59	18298/18	N	P	F	1.4	1	PT/VLBW	poor sucking	10000	0	5950	1	900	1	0.15	1	0.17	0	NO	0	2.2	0	3	positive	Positive	0
60	18305/18	N	P	M	1.6	1	PT/LBW/PROM	poor sucking	4400	1	2398	0	110	0	0.04	0	0.04	0	yes	1	2.4	0	2	positive	negative	0

61	18329/18	C	T	F	2.3	3	LBW/PROM	Lathargic/Grunt	32000	1	26080	1	2560	1	0.09	0	0.1	0	NO	0	3.5	0	3	negative	negative	0
62	18334/18	C	T	M	3.1	4	PROM	poor sucking	28000	1	24360	1	2940	1	0.12	1	0.13	0	NO	0	3	0	4	negative	negative	2
63	18349/18	N	P	F	1.8	2	PT/LBW	Lathargic/Grunt	17600	0	11968	1	1584	1	0.13	1	0.15	0	yes	1	2.4	0	4	negative	Positive	1
64	18366/18	N	P	M	2	1	PT/LBW	poor sucking	12000	0	10080	1	1080	1	0.1	0	0.12	0	NO	0	1.5	1	3	negative	negative	4
65	18373/18	N	P	F	0.95	1	PT/ELBW/PPROM	ICR+/PS /FEVER	40000	1	33600	1	6400	1	0.19	1	0.23	0	NO	0	2	0	4	negative	negative	0
66	18382/18	N	P	M	2.6	2	PT	poor sucking	20900	0	15675	1	3135	1	0.2	1	0.25	1	NO	0	1.8	0	4	negative	negative	1
67	18392/18	C	T	M	2.3	1	LBW/PROM	PS/ICR	16000	0	10640	1	1680	1	0.15	1	0.18	0	yes	1	3.2	0	4	negative	negative	0
68	18500/18	N	T	M	2.4	2	LBW	poor sucking	24000	0	16080	1	840	1	0.05	0	0.05	0	NO	0	1.2	1	3	negative	negative	3
69	18528/18	N	T	F	3.2	1	H/o chicken pox	poor sucking	11600	0	8004	1	1218	1	0.15	1	0.17	0	NO	0	1.1	1	4	positive	Positive	0
70	18632/18	/Vaccu	T	M	3	1	Vaccum	Fever/Grunt/PS	14000	0	11200	1	4410	1	0.39	1	0.64	1	YES	1	2.5	0	5	negative	Positive	10
71	18726/18	N	P	M	2.3	3	PT/LBW/PROM	poor sucking/Fever	20000	0	17000	1	5000	1	0.29	1	0.41	1	yes	1	3.6	0	5	negative	Positive	5
72	18822/18	N	P	F	1.5	1	PT/LBW	poor sucking	20000	0	16100	1	2000	1	0.12	1	0.14	0	NO	0	2.7	0	3	negative	negative	3
73	18917/18	C	T	F	2.7	3		pink,alert	22000	0	15950	1	1650	1	0.1	0	0.11	0	NO	0	3.5	0	3	negative	negative	5
74	18964/18	C	T	F	3.2	6		pink,alert	10900	0	3270	1	654	1	0.2	1	0.25	1	NO	0	3.8	0	4	negative	negative	1
75	19115/18	N	T	M	2.2	3	PT/LBW	poor sucking	22000	0	11330	1	1540	1	0.13	1	0.15	1	yes	1	1.9	0	4	negative	negative	5
76	19139/18	C	T	M	3	1		Lathargic/Grunt	13200	0	4752	1	132	0	0.02	0	0.02	0	yes	1	0.46	1	3	positive	negative	2
77	19171/18	C	P	M	1.9	1	PT/LBW	ICR+/PS	24000	0	18480	1	2400	1	0.12	1	0.14	0	yes	1	3.5	0	4	negative	Positive	0
78	19172/18	C	P	F	2	1	PT/LBW	ICR+/PS	18000	0	14040	1	1080	1	0.07	0	0.08	0	NO	0	3	0	2	negative	negative	1
79	19173/18	C	P	F	1.7	1	PT/LBW	Lathargic/Grunt	20000	0	15500	1	1900	1	0.12	1	0.13	0	NO	0	2.8	0	3	negative	negative	1
80	19187/18	C	P	F	2.2	1	PT/LBW	Lathargic/Grunt	16200	0	16038	1	486	0	0.03	0	0.03	0	NO	0	3.3	0	1	negative	negative	2
81	19229/18	N	P	M	2.2	2	PT/LBW	ICR+/PS/Fever	7700	0	6699	1	962	1	0.14	1	0.16	0	YES	1	1.6	0	4	negative	negative	11
82	19246/18	C	P	M	2.4	3	PT/LBW	ICR+/PS	18700	0	15708	1	1963	1	0.12	1	0.14	0	yes	1	1.6	0	5	negative	negative	2
83	19359/18	C	T	F	3.4	3		poor sucking/Grunt	28200	1	24534	1	3384	1	0.13	1	0.16	0	NO	0	0.78	1	5	negative	Positive	0
84	19411/18	N	P	M	2.3	1	PT/LBW	poor sucking	19200	0	17664	1	864	1	0.04	0	0.05	0	YES	1	1.7	0	3	negative	negative	0
85	20213/18	C	T	M	3.4	3	PROM	poor sucking/Grunt	28000	1	18620	1	5880	1	0.31	1	0.41	1	yes	1	4.5	0	6	positive	Positive	9
86	20583/18	C	P	M	1	1	PT/ELBW	Lathergic/Grunt	23600	0	15340	1	1062	1	0.06	0	0.07	0	NO	0	1.6	0	2	negative	negative	5
87	21306/18	N	P	M	2.3	1	PT/LBW	poor sucking	11600	0	13108	1	870	1	0.06	0	0.13	0	yes	1	2.8	0	3	negative	negative	11
88	21832/18	N	T	F	3.2	3	Prolonged Labour	ICR+/PS	24000	0	20040	1	2640	1	0.13	1	0.15	0	yes	1	2.5	0	4	negative	negative	2
89	22184/18	C	P	M	2	1	PT/LBW	poor sucking	11500	0	8855	1	1380	1	0.15	1	0.18	0	NO	0	2.7	0	3	positive	Positive	4
90	22611/18	N	P	F	1	1	PT/ELBW/PPROM	poor sucking	30200	1	12080	1	1208	1	0.1	0	0.06	0	NO	0	2.5	0	3	negative	negative	1
91	23688/18	C	T	F	3.2	1	CPD/BMV	Lathergic	12000	0	8400	1	840	1	0.1	0	0.11	0	NO	0	3	0	2	negative	negative	0
92	23912/18	C	T	M	2	1	LBW	poor sucking	57000	1	45030	1	4845	1	0.1	0	0.12	0	NO	0	8	0	3	negative	negative	2

93	24155/18	N	P	F	1.5	1	preterm/VLBW	Lathergic/ps	10500	0	3832	1	892	1	0.23	1	0.03	1	yes	1	2.4	0	5	negative	Positive	0
94	24492/18	C	P	F	2.3	4	preterm/LBW	poor sucking	54500	1	42782	1	5450	1	0.12	1	0.14	0	yes	1	0.51	1	6	negative	Positive	15
95	24939/18	C	P	F	2.5	1	preterm/LBW	good sucking	17500	0	11812	1	2100	1	0.17	1	0.21	0	NO	0	3.6	0	3	negative	negative	1
96	25162/18	C	T	F	3.6	3	-	poor sucking	20400	0	14790	1	4590	1	0.31	1	0.45	1	NO	0	3	0	4	negative	negative	4
97	25611/18	C	P	M	2.3	3	preterm/LBW	pink,alert	13000	0	9425	1	1365	1	0.14	1	0.16	0	NO	0	2.5	0	3	negative	negative	6
98	25612/18	C	P	M	2.2	3	preterm/LBW	pink,alert	12400	0	9300	1	1302	1	0.14	1	0.16	0	NO	0	3.4	0	3	negative	negative	6
99	25613/18	C	P	M	1.9	3	preterm/LBW	good sucking	12800	0	9088	1	1024	1	0.11	0	0.12	0	NO	0	1.9	0	2	negative	negative	5
100	25620/18	N	T	M	2.7	6		poor sucking/Grunt	19000	0	13300	1	4560	1	0.34	1	0.52	1	yes	1	1.3	1	6	positive	Positive	8

N - Normal C- caesarian T - Term P- Preterm M - Male F - Female PS- Poor Sucking ICR- Inter Costal Rectraction